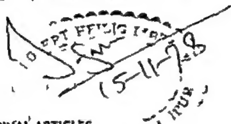
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CONGENITAL HYPOTHYROIDISM IN SWEDEN

Incidence and Age at Diagnosis

J ALM A LARSSON and R ZETTERSTRÖM

From the Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Alm J Larsson A and Zetterstrom R (Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) Congenital hypothyroidism in Sweden Incidence and age at diagnosis Acta Paediatr Scand 67 1 1978.—A total number of 112 children with congenital hypothyroidism were diagnosed in all Children s Hospitals and Pediatric Wards in Sweden during the 7 year period 1969–1975 Since it may be assumed that all cases of congenital hypothyroidism which were diagnosed during that period were seen in one of these hospitals the incidence of congenital hypothyroidism in Sweden can be calculated to be 1 6900 live births In spite of an efficient National Health Care Program for Infants the diagnosis was delayed until after an age of three months in 52% of the cases This fact supports the view that mass screening of newborns for congenital hypothyroidism has to be introduced in Sweden However the beneficial effects of such a program cannot be fully elucidated until it has been considered whether earlier instituted treatment would have improved the outcome of children in whom a diagnosis was made after 3 months of age

KEY WORDS Congenital hypothyroidism congenital cretinism

Thyroid hormone is essential for normal somatic and psycho-motor development Congenital hypothyroidism (CH) therefore has to be diagnosed as early as possible so that substitution therapy can be initiated before irreversible postnatal brain damage has occurred A correlation has been found between the age at which treatment is started and the prognosis (4 6 7) Early diagnosis may however be difficult to make since the clinical symptoms of CH in the newborn very often are nonspecific (9) To facilitate detection of children with CH programs for mass screening of newborn infants for CH have been started in different countries (1 3 5 11) In this study we have estimated the incidence and age at diagnosis of children with CH in Sweden in order to provide background data for a decision on mass screening

MATERIALS AND METHODS

In 1976 a questionnaire was sent out to all children s hospitals and pediatric wards in Sweden (n=44) requesting information about all patients with hypothyroidism diagnosed during a seven year period (1969–1975) Data pertaining to the clinical and laboratory findings in all of the patients was obtained The diagnostic criteria for CH in this study was a diagnosis of hypothyroidism before the age of two years

RESULTS

Replies were received from all 44 departments A total number of 168 patients with hypothyroidism diagnosed from 1969 to 1975 was reported Of the 168 hypothyroid children 112 i e 67 girls and 45 boys were diagnosed before the age of two years and were classified as CH In all 112 patients hypothyroidism was confirmed by thyroid function tests before therapy was started In three chil

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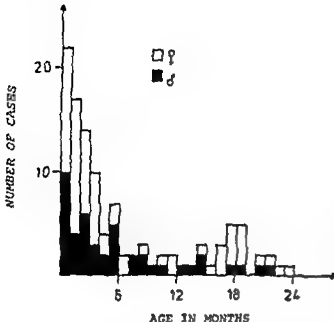


Fig 1 Age at a time of diagnosis of those 112 cases of congenital hypothyroidism which were diagnosed in Sweden during the seven year period 1969–1975 In 52% of the cases a correct diagnosis was not made until after an age of 3 months

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The age at onset of symptoms compatible with hypothyroidism could be evaluated in 84 of the 112 children with CH Of these 76 (91%) had one or more symptoms before the age of three months The most frequently occurring clinical signs were obstipation and lethargy with feeding difficulties

According to the vital statistics in Sweden (10) 767 698 children were born during the seven year period from 1969 to 1975 The incidence of CH thus can be calculated to be 1 in 6854 live births

DISCUSSION

The incidence of CH in the present investigation was 1 in 6900 which is in good accordance with the value which was obtained in a similar study in the Netherlands, i e , 1 in 6300 (2) and with that in a large screening program in Canada (3), i e , 1 in 6000

Several factors may have influenced the incidence of CH in this study, e g the question whether all cases of CH diagnosed between 1969 and 1975 were reported Furthermore all patients with hypothyroidism diagnosed before the age of two years were classified as having CH We were therefore unable to exclude that some of the 112 children with CH had acquired hypothyroidism, a condition, which, however, is regarded to be very rare in children less than two years of age Consequently the estimated incidence of CH in Sweden, 1 in 6900, most likely is not too high but may be an underestimate, since the diagnosis may have been missed in some cases

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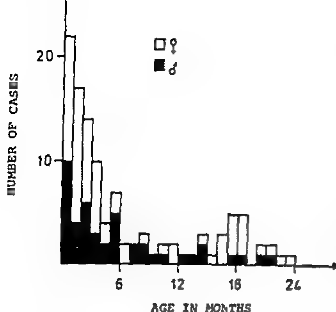


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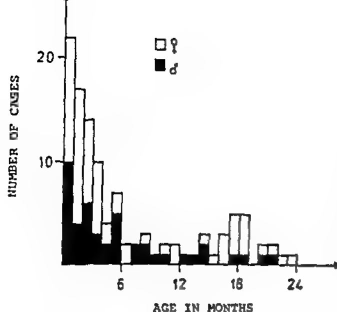


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DIALYSIS AND RENAL TRANSPLANTATION OF CHILDREN IN EUROPE 1975[1]

C CHANTLER K SCHARER G GILLI F P BRUNNER H J GURLAND
C JACOBS N H SELWOOD and A J WING

From the Registration Committee of the European Dialysis and Transplant Association London Heidelberg Basle Munich Paris and Bristol

ABSTRACT Chantler C Scharer K Gilli G Brunner F P Gurland H J Jacobs C Selwood N H and Wing A J (Registration Committee of the European Dialysis and Transplant Association) Dialysis and transplantation of children in Europe 1975 Acta Paediatr Scand 67 5 1978 —The number of new paediatric patients accepted for treatment by regular dialysis and transplantation increased more slowly than in previous years Survival in children above 10 years appeared to be better with all modes of therapy than in younger children The only improvement in survival noted among the different treatments was in patient and graft survival of living donor transplants A quarter of all children transferred to home dialysis were less than 10 years of age Nephronophthisis and Henoch Schonlein nephritis emerged as major primary renal diseases In 1975 the proportion of retransplants in children rose and living donor grafts from fathers were more common than from mothers Evening dialysis was practised more frequently in both hospital and home dialysis and rehabilitation in these patients seemed to be better than for those dialysed at other times Renal osteodystrophy was present in at least half of all children dialysed for more than 1 year The degree of growth retardation was affected by sex chronological age and the primary renal disease Body height on dialysis and after transplantation progressively reduced in the majority of children Growth seemed to be more impaired in boys than in girls on dialysis Bone age advanced faster than height age especially in girls The pubertal growth spurt was usually delayed and depressed on long term dialysis and the development of genitalia and pubic hair as well as menarche was retarded

KEY WORDS Chronic renal failure dialysis transplantation osteodystrophy growth failure pubertal development

This paper provides a summary of the latest data of the European Dialysis and Transplant Association (EDTA) on the children treated by regular dialysis and renal transplantation in Europe up to the end of 1975 (1 2) It follows similar reports published earlier in this journal (3) and is supplemented by the results of a special survey on growth skeletal maturation and osteodystrophy organized among selected dialysis centres

At the end of 1975 the total number of patients on the Paediatric Register of the EDTA was 1111 of whom 835 were alive The increasing number of children alive on regular dialysis or after successful transplantation in Europe is demonstrated in Fig 1 More new children started treatment in 1975 than in previous years but the rate of increase decreased and the number of first transplants diminished from 1974 to 1975 (Table 1) As in the previous year Denmark France and the Netherlands were the countries with the highest proportion of children on treatment (Fig 2) During 1975 only 2 countries accepted more than 1 child per million total population suggesting that

[1] Extract of a Report from the Registry of the European Dialysis and Transplant Association (EDTA) by kind permission of the Council of the EDTA

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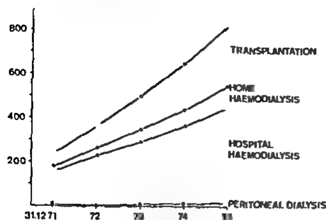


Fig 1 Paediatric patients alive 1971–1975

Table 1 *New children admitted to regular dialysis and renal transplantation*

	1972	1973	1974	1975
Patients instituted into hospital haemodialysis		133	156	172
Patients instituted into home haemodialysis		25	29	37
Patients receiving first transplant		78	114	102
New patients starting initial treatment by dialysis or trans plantation	157	175	225	230

provision of facilities for treatment of end stage kidney failure in suitable children is still not a priority in most countries

The *age* at start of therapy was similar to last year only five (2%) of all new children entering treatment in 1975 being under 5 years and 32% under 10 years

At the end of 1975 290 *centres* were treating paediatric patients Fifty three per cent of all new children were treated by the 26 paediatric centres which accepted 3 or more children Only 13 of the paediatric centres were treating 10 or more children at the end of 1975 and one way to improve the provision of facilities for children in terminal renal failure might be for these centres to be enabled to increase their activity

Patient survival was found to be similar to that reported last year Four years after start of therapy it was 89% for home dialysis 70% for hospital dialysis, 80% after a living donor and 70% after a cadaver transplantation (Fig 3) The survival of children on hospital dialysis at 2 to 3 years was 3–4% less in children treated during 1973–1975 compared to all children on the Register *A significant im*provement was noted only for patients after a living donor transplantation Graft survival for first living donor grafts was 69 and 56% and for first cadaver grafts 54 and 45% at two and four years respectively

Age differences in survival became apparent soon after treatment commenced At 3 years survival on hospital dialysis was 63% 68% 77% and 73% for the age groups 0 to 5 5 to 10 10 to 15 and 15 to 34 years respectively

Living donor grafts had a 16% better survival at 2 years in children age 10–15 than in younger patients whereas in cadaver grafts the difference was only 3% Of 19 children who commenced treatment before 1967 10 were still alive and one was lost to follow up at the end of 1975 (Fig 4) suggesting that long survi

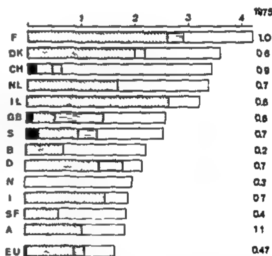


Fig 2 Treated children per million total population alive 31 12 1975 (as horizontal bars) The number of new paediatric patients per million population commencing treatment by regular dialysis or transplantation in 1975 is indicated at the right hand of the figure ■ Peritoneal dialysis □ hospital haemodialysis ▤ home haemodialysis □ transplantation F=France DK=Denmark CH=Sweitzerland NL=Netherlands IL=Israel GB=United Kingdom S=Sweden B=Belgium D (BRD)=Federal Republic of Germany N=Norway I=Italy SF=Finland A=Austria EU=total Europe

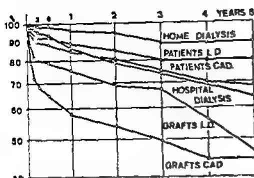


Fig 3 Cumulative patient survival of paediatric patients on home haemodialysis hospital haemodialysis and after a living donor (*L D*) or cadaver (*CAD*) transplant At the bottom graft survival after L D and CAD transplantation Life table method

val is obtainable for a majority of children which should enable a substantial proportion to reach adult life

The practice of *home dialysis* training was restricted mainly to a few centres in the United Kingdom the Federal Republic of Germany and France only ten centres have transferred 3 or more dialysed children to the home A quarter of all children trensferred in 1975 were below ten years (Fig 5)

The number of new *first transplants* de creased from 111 in 1974 to 102 in 1975 with the proportion of living donors remaining about the same (20%) 3 mothers 15 fathers 2 obligatory nephrectomies Retransplants (24 in 1975) were more than in previous years

This year *primary renal diseases* were sub divided into more categories It was apparent that juvenile nephronophthisis and Hen och Schönlein nephritis were major causes of renal failure (Table 2)

The time of the day when dialysis was un dertaken changed with a rise in the performance of evening dialysis in hospital and especially in home treated patients (13% and 54% of all patients dialysed in 1975 re spectively) A continuing trend to dispense with non-disposable dialysers was noted

The *vascular access* for haemodialysis [illegible] tions from country to country

Internal arteriovenous fistulae were most fre quently used followed by external Scribner shunts (17%) and different types of vascular grafts (8%) The use of external shunts de creased from 32% in the 5 to 8 year olds to 16% in the 12 to 15 year olds However all children below 5 years had fistulae or a vascu lar graft The *single needle technique* was practised occasionally in 12% and routinely in 20% of paediatric patients on haemodialysis with some type of fistula as regular access in 1975

Hepatitis continued to be a clinical problem 24% of all centres having had at least one HB Ag positive child in 1975

Rehabilitation was assessed according to the ability to attend school Full school activ ity in ordinary school at home or in special institutions was achieved by 57 5% of all children on hospital dialysis and 85 to 87% on home dialysis or after transplantation The poorer school attendance of hospital dialysed patients may in part be due to inadequate provision of special hospital schooling whilst on treatment The remarkable increase in evening as opposed to night dialysis observed on home dialysis (from 30% in 1974 to 54% in 1975) was associated with a slightly better school at tendance suggesting that more frequent eve ning dialysis in children may enable a more normal life

Body growth in dialysed and transplanted

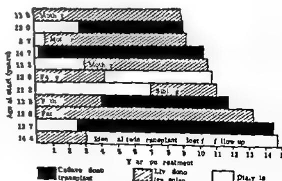


Fig 4 Modes of treatment employed in 11 surviving children treated before 1967 Age at the start of treatment is shown on the vertical axis

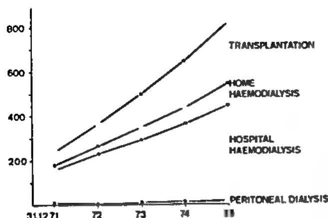


Fig 1 Paediatric patients alive 1971–1975

Table 1 *New children admitted to regular dialysis and renal transplantation*

	1972	1973	1974	1975
Patients instituted into hospital haemodialysis		133	156	172
Patients instituted into home haemodialysis		25	29	37
Patients receiving first transplant		78	114	102
New patients starting initial treatment by dialysis or transplantation	157	175	225	230

provision of facilities for treatment of end stage kidney failure in suitable children is still not a priority in most countries

The *age* at start of therapy was similar to last year only five (2%) of all new children entering treatment in 1975 being under 5 years and 32% under 10 years

At the end of 1975, 290 *centres* were treating paediatric patients Fifty three per cent of all new children were treated by the 26 paediatric centres which accepted 3 or more children Only 13 of the paediatric centres were treating 10 or more children at the end of 1975 and one way to improve the provision of facilities for children in terminal renal failure might be for these centres to be enabled to increase their activity

Patient survival was found to be similar to that reported last year Four years after start of therapy it was 89% for home dialysis 70% for hospital dialysis, 80% after a living donor and 70% after a cadaver transplantation (Fig 3) The survival of children on hospital dialysis at 2 to 3 years was 3–4% less in children treated during 1973–1975 compared to all children on the Register A significant improvement was noted only for patients after a living donor transplantation Graft survival for first living donor grafts was 69 and 56% and for first cadaver grafts 54 and 45% at two and four years respectively

Age differences in survival became apparent soon after treatment commenced At 3 years survival on hospital dialysis was 63%, 68% 77% and 73% for the age groups 0 to 5, 5 to 10 10 to 15 and 15 to 34 years, respectively

Living donor grafts had a 16% better survival at 2 years in children age 10–15 than in younger patients whereas in cadaver grafts the difference was only 3% Of 19 children who commenced treatment before 1967 10 were still alive and one was lost to follow up at the end of 1975 (Fig 4) suggesting that long survi

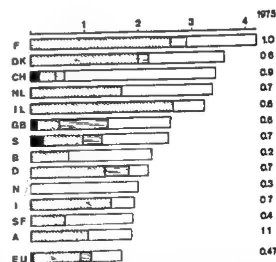


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lowed for 2 years or more the number of patients with an increase in their height percentile increased in only 3 out of 31 boys but in 14 out of 37 girls with the same kidney disorders

Growth velocity after transplantation also showed changes according to sex In boys up to age 16 followed for 1 year or longer mean absolute growth rate was 2 9 cm per year after transplantation (compared to 2 6 cm per year on dialysis) and in transplanted girls only 2 2 cm per year (compared to 2 6 on dialysis) The height percentile improved in only 5 of 23 children transplanted successfully for more than 3 years Growth after transplantation seemed to be affected more in children above age 12 than in younger patients (Fig 7)

In 24 patients body height was measured for 1 year or more on dialysis and successively for 1 year or more after transplantation In 6 out of 13 boys and 4 out of 11 girls absolute growth velocity increased after transplantation

Skeletal maturation at start of treatment was found to be retarded to a similar degree as height and the delay usually increased on long term dialysis In most children especially in girls the increase in bone age from the start

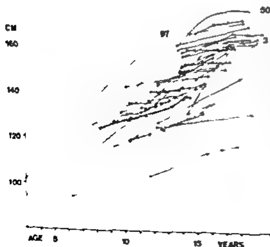


Fig 6 Height of 43 boys on haemodialysis followed for longer than 3 years

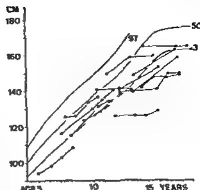


Fig 7 Height of 14 boys followed for longer than 3 years after transplantation

of dialysis to the last observation was higher than the corresponding increase in height age It seems therefore that the growth potential will decrease as treatment continues The assessment of bone age was also used as a reference for growth velocity In 29 boys followed for 1 year or more on dialysis or after transplantation up to age 16 two thirds fell below the third percentile for the corresponding bone age

The delay in *pubertal development* of dialysed and transplanted children suggested by the growth data was confirmed from the data on the pubertal development of 172 patients analysed according to Tanner & Whitehouse (4) About a quarter of all dialysed boys failed to show any development of genitalia or pubic hair before age 14 years i e the latest age when these signs usually appear in normal children Almost half of the dialysed girls did not show any breast development before age 13 or menarche before age 15 i e the latest moment of normal appearance of these signs Secondary sex characteristics (stage 2 to 4) did appear in about half of the dialysed boys and girls but were delayed compared to normal children

ACKNOWLEDGEMENTS

The Registration Committee of the EDTA is supported by grants from Governments National Societies of Nephrology the EDTA the European Society for Paediatric

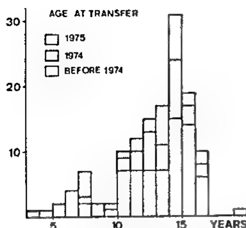


Fig 5 Age at transfer to home dialysis in 129 paediatric patients The year of transfer is indicated by different bars

children was analysed longitudinally for children on the same treatment over a long period using the growth charts given by Tanner et al (4) Fig 6 shows the results of repetitive height measurements in a group of 43 boys followed for at least 3 years *on haemodialysis* Most children fell progressively away from their height percentile at the start of treatment When the last measurement was compared with that at start of dialysis the height decreased in the average from −1 8 to −3 2 S D in boys and from −1 6 to −2 6 S D in girls Only 7% of the boys but 24% of the girls improved their percentile

The prognosis of the growth failure in dialysed children appeared somewhat better when *growth velocity* was compared at repetitive intervals The absolute growth velocity increased after treatment for longer than 2 years in 48% of all analysed patients and in about two thirds of children growth velocity related to percentiles for age increased during long term dialysis This increase in growth velocity is probably accounted for by the late maturation of these children This observation is in agreement with our finding that continued growth was noted in a third of all patients above age 18 years in males and age 16 in females i e beyond the time of epiphyseal closure in the normal population Unfortunately the late growth achieved does not compensate for that already lost in earlier years

The proportion of children below the third percentile for height and followed for 2 years or more increased from 35% to 70% in patients with glomerulonephritis, and from 56% to 88% in those with hereditary or congenital kidney disorders when the height at start of dialysis was compared with that of the last observation The sex dependance of growth velocity on dialysis mentioned above was also noted in a restricted group of patients with glomerulonephritis and pyelonephritis fol

Table 2 *Primary renal disease in children accepted for treatment in 1975*

	No of patients		%
Glomerulonephritis (G N) histologically classified	31		13 5
Membrano proliferative G N		11	4 8
Focal segmental sclerosis		7	3 0
Proliferative G N (intra and extracapillary with extensive crescents rapidly progressive)		5	2 2
Proliferative G N mesangial endocapillary and other types		5	2 2
Chronic G N sclerosing and other types		3	1 3
G N without histological examination	38		16 5
Henoch Schonlein nephritis	17		7 4
Lupus erythematosus	5		2 2
Pyelonephritis	43		18 7
Medullary cystic disease nephronophthisis	24		10 4
Cystic kidney disease other types and unspecified	3		1 3
Cystinosis	7		3 0
Alport s syndrome	3		1 3
Hereditary nephropathy unspecified	4		1 7
Oligomeganephronic hypoplasia	3		1 3
Segmental renal hypoplasia (Ask Upmark kidney)	6		2 6
Congenital renal hypoplasia unspecified	14		6 1
Renal vascular disease	1		0 4
Cortical tubular necrosis	1		0 4
Drug induced nephropathy	1		0 4
Traumatic loss of kidneys	1		0 4
Others and unknown	27		11 7
Not recorded	1		0 4
Total	230		100

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FUNCTION AND DIMENSIONS OF THE CIRCULATORY SYSTEM IN ANOREXIA NERVOSA

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ABSTRACT Fohlin L Freyschuss U Bjarke B Davies [redacted] T M and Thoren C (Department of Paediatrics Karolinska Institutet St Goran s Hospital Stockholm Sweden) Function and dimensions of the circulatory system in anorexia nervosa Acta Paediatr Scand [redacted] 11 1978.—The functional and dimensional components of the oxygen transporting system was studied in 17 female and 11 male patients suffering from anorexia nervosa Both groups were [redacted] 9 years old on average and had lost about 25% of their weight Measurements at rest included blood and heart volume heart rate blood pressure oxygen uptake ($\dot{V}O_2$) RQ blood lactate (LA) and in 6 of the patients cardiac output During bicycle ergometry the determinations of heart rate blood pressure LA $\dot{V}O_2$ and cardiac output were repeated and maximal aerobic power was determined A low metabolic rate with bradycardia and hypotension was apparent at rest Blood and heart volume was decreased proportionally [redacted] the weight loss On a given work load $\dot{V}O_2$ was lowered [redacted] the same extent as the resting metabolic rate At maximal effort $\dot{V}O_2$ was reduced out of proportion to the circulatory dimensions and maximal heart rate was low During exercise cardiac output was normally related to $\dot{V}O_2$ and stroke volume was maintained indicating a normokinetic circulation and an unimpaired myocardial function The main cause of the low maximal aerobic power seems to be the reduced muscle mass

KEY WORDS Blood volume blood lactate cardiac output children heart volume maximal exercise oxygen uptake

In anorexia nervosa (AN) symptoms from the cardiovascular system are often apparent such as bradycardia hypotension and acrocyanosis (8 28) The presence of unspecific ventricular repolarization changes on the ECG (23 27) have evoked questions of the myocardial involvement in the disease Although many patients have a hyperactive behaviour and cardiovascular symptoms are common there are no data in the literature regarding function of the circulatory system in anorexia nervosa The potentially very poor prognosis—a mortality rate of about 10% being reported—and unexpected sudden deaths (6 21 24 28) initiated a series of studies (1 10 11 14–16) to evaluate the cardiovascular accommodation to the long standing caloric starvation in AN patients In the present study various dimensional components of the oxygen transporting system have been examined at rest and during submaximal and maximal exercise performance

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MATERIALS AND METHODS

Seventeen female and 11 male patients were studied after the informed consent by the patients as well as by their parents and after the approval by the Ethical Committee of Karolinska Institutet The patients selected for the investigation all conformed to the following criteria chiefly from Dally (8)

1 age at onset less than 25 years
2 active refusal to eat with accompanying pronounced weight loss

Nephrology and firms listed in the full Combined Report on Regular Dialysis and Transplantation of Children in Europe 1975 (2) We are grateful to the editors and publishers for allowing us to present this report

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Table 3 *Circulatory and metabolic data of patients with AN at rest and during maximal exercise*
Mean values ±S D are given Within parentheses numbers of patients when fewer than the whole series

	Rest		Maximal work	
	Girls (n=17)	Boys (n=11)	Girls (n=13)	Boys (n=8)
Load W			99±13 2	113±?5 5
Oxygen uptake l STPD×min⁻¹	[illegible] 139±0 018 (12)	0 153±0 037 (6)	1 21±0 [illegible]	1 38±0 33
Oxygen uptake per kg body weight ml STPD×kg⁻¹			3?±5	35±3
R	0 81±0 10 (12)	0 82±0 06 (6)	1 14±0 07	1 18±0 07
Blood lactate mmol×l⁻¹	1 7±0 9 (11)	1 8±1 2 (7)	11 8±2 2	12 6±2 1
Heart rate b p m	*53±9 3*	*5?±11 7*	*174±8 2*	*175±5 7*
Blood pressure mmHg				
syst	97±9 ?	96±10 0	145±20 9 (9)	149±17 4 (6)
diast	65±5 2 (15)	67±6 0		
Minute volume of ventilation per l oxygen uptake			*41 8±7 08*	*39 1±6 30*

was in both girls 75 ml and boys 80 ml within the limits of healthy children as determined by the total hemoglobin method (18) For a given body weight heart volume was within the range expected for healthy children (14 26) except in 5 of the patients (Fig 1) However in the relationship the majority of points were above the regression line In the AN children the relation between blood volume and heart volume was highly significant (r= 0 80 p<0 001) The oxygen uptake at rest was on the average about 20% lower than predicted from sex age and body surface area and both girls and boys showed evidence of bradycardia 53 and 52 b p m and hypotension 97/65 and 96/67 mmHg respectively Three *patients had shortly before this examination an* extreme bradycardia of about 30 beats per minute Sinus rhythm dominated but periods of sinus arrest and ectopic atrial rhythm were recorded The mean values of blood lactate 1 8 mmol ×l⁻¹ were within the upper normal range of the analysing laboratory (26)

During exercise the oxygen uptake for a given work load was lower than expected (Fig 2) the regression line was displaced parallel to and below the normal relationship (3) $\dot{V}O_{2\,max}$ averaged 1 21 ×min⁻¹ in the girls

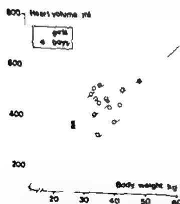


Fig 1 Heart volume [illegible] relation [illegible] body weight in ‿6 children with AN (Y=59 88+10 49x r=0 78) The regression line ± [illegible]D of 109 healthy children (Y= 61 93+10 06x r=0 86)(6)

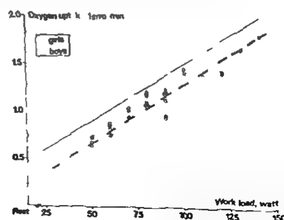


Fig 2 Oxygen uptake of various work loads in ‿8 AN patients represents AN (Y=0 ???+0 0105x r= 0 85 n=46) —denotes regr line [illegible]f healthy children (3)

Table 1 *Physical characteristics of 17 female and 11 male anorexic children*

Individual data and mean values ±S D of the girls and boys are given

	Age (yrs)	Weight (kg)	Height (cm)	Per cent weight loss	Duration (yrs)
Girls					
H H	12 1	30 0	153	15 0	0 5
G Z	12 9	24 5	153	30 0	0 5
C W	13 1	39 0	163	35 0	0 5
H Hj	13 2	40 5	168	19 3	0 9
[illegible] A	13 3	33 5	157	14 1	0 5
M E	13 6	48 5	169	21 8	0 8
M St	14 0	35 0	164	27 1	0 6
C P	14 2	34 5	157	14 8	1 3
A S	14 8	42 9	173	24 5	1 0
G G	15 2	34 0	158	39 8	1 5
K Å	16 7	38 0	173	32 0	2 0
A H	15 9	35 8	164	33 7	1 5
Y [illegible]	16 6	48 2	169	25 9	2 0
P W	16 7	35 2	166	30 5	1 0
U N	16 9	41 8	180	25 9	1 0
L P	17 6	42 5	162	24 1	0 6
C A	17 7	40 5	163	32 5	3 0
Mean	14 9	37 8	164 2	26 2	1 1
S D ±	1 8	6 1	7 4	7 5	0 7
Boys					
M M	11 3	28 6	142	37 8	2 0
J W	12 0	27 3	146	20 4	0 9
L K	12 5	31 0	156	20 5	1 0
B G	13 7	50 6	188	24 5	1 0
P W	14 0	38 5	164	23 0	0 8
T [illegible]	15 2	36 6	164	22 1	0 5
T L	15 9	36 3	164	30 0	1 0
T T	16 0	56 7	187	22 2	0 8
M S	17 1	51 0	184	22 0	1 0
U P	17 5	49 0	184	28 0	1 0
A N	18 2	48 7	186	21 5	1 0
Mean	14 8	41 3	169 6	24 7	1 0
S D ±	2 3	10 3	17 1	5 3	0 4

3 no evidence of schizophrenia severe depression or organic disease

The psychiatric condition of patients varied but none was primarily psychotic

Amenorrhoea was present in all the postpubertal girls Four girls had not passed menarche when they started to lose weight and 3 boys had no signs of puberty Mean age of both the girls and boys was 14 9 years

The physical characteristics of the AN patients and duration of the weight loss are given in Table 1 The body weight in all except 4 was less than −2 S D on the growth chart relating body weight to height their average weight loss was about 25% None had edema or anemia and blood electrolytes were within normal limits One boy later died due to septicemia following intravenous nutrition

The investigation was performed while the patients were treated at St Goran s Children s Hospital Three patients were on drug therapy (Thioridazin chloride Mallorol®) The study was completed during the years 1972–76

The patients were measured in accordance with standard procedures for body weight (kg) using a clinical balance accurate to ±0 1 kg and height (cm) using a stadiometer accurate to ±0 5 cm Blood volume was determined with ^{125}I labelled albumin (29) plasma volume being derived from blood volume and hematocrit determinations The heart volume (HV ml) was measured in the prone position (20) Expiratory gas was collected in Douglas bags and the volumes were measured with a Tissot spirometer Gas samples were analysed for oxygen and carbon dioxide by the micro-Scholander technique Heart rate (HR) was obtained from ECG Blood lactate (LA) was determined enzymatically (7) from arterialized (finger prick) blood Cardiac output was determined by the dye dilution technique with indocyanine green (Cardio-Green®) as the indicator and a Beckman densitometer as recording unit Resting measurements were taken in supine position Exercise was performed in the sitting position on an electrically braked bicycle ergometer (Elema) at a pedalling rate of 60 rpm During exercise HR was measured every minute minute volume of ventilation and oxygen uptake were determined during the third–sixth minute of the work load The exercise tests were performed with a stepwise increase of loads which usually comprised two submaximal and one maximal load It was not always possible to apply the levelling off criteria of $\dot{V}O_2$ max The patients were simply encouraged to pedal the bicycle to exhaustion The secondary criteria of blood lactate and respiratory quotient (2 3) were used as evidence of maximal effort Seven failed to fulfil these criteria

RESULTS

Mean values of the circulatory and metabolic data measured at rest before the exercise test in the AN children are summarized in Tables 2 and 3 The blood volume per kg body weight

Table 2 *Circulatory data of the anorexic children obtained at rest*

Mean values ±S D are presented Within parentheses are numbers of patients when fewer than the whole series

	Girls (n=17)	Boys (n=11)
Heart volume ml	437±74 2	547±135 9 (9)
Hemoglobin g×l⁻¹	138±12 6	135±10 5
Blood volume l	2 79±0 47 (14)	3 26±0 8?
Blood volume/body weight ml ×kg⁻¹	75±11 8 (14)	80±13 5

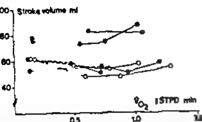


Fig 5 Stroke volume in relation to oxygen uptake at rest supine and during upright exercise in 4 AN boys and 2 AN girls Symbols as in Fig 1

tion was of the same order as that seen at rest A low body temperature and metabolic rate are constant findings in anorexia nervosa and as hypothermia is associated with a low heart rate (9 22) this may be a factor contributing to the lowered heart frequency observed

Cardiac output at rest and during work was within 1 S D and −2 S D of the values found in 13–14 year old boys (12) and it was normally related to oxyen uptake Stroke volume was well maintained during work These findings indicate a normokinetic circulation and an unimpaired myocardial function At roughly similar levels of oxygen uptake during exercise stroke volumes in 2 of the AN boys were within ±S D of the values found in healthy boys (12) while values below the lower normal range was observed in 2 AN boys and 2 AN girls When similarly compared with another series of healthy boys (13) all stroke volumes were within ±S D of the reference values The relation between stroke volume and blood volume varied between 1 85 and 2 30 and hence similar to corresponding ratios found in healthy adults (17) in turn indicating all an ordinary adaptation of the stroke volume to the circulating blood volume In 5 of the 6 AN children in whom cardiac output was determined ECG was normal both at rest and during exercise the sixth patient had at one occasion unspecific ST T changes which remained unchanged during exercise within 10 days ECG had normalized

In 6 other patients T wave inversions were recorded in the precordial leads during exercise These changes became however less marked with increasing work loads Further during a preceding orthostatic test a similar ECG pattern was observed and it could be abolished by the administration of a β receptor blocking agent (Inderal®) It therefore seems probable that these repolarization changes were of functional origin

For a given absolute work load the oxygen uptake was significantly reduced This might lead one to suspect that AN children pedal with greater mechanical efficiency than normal but the regression line between oxygen uptake and load reveals a parallel displacement of the same magnitude as their resting metabolic rate

The major effect of anorexia nervosa on physical performance is undoubtedly a reduction in maximal aerobic capacity with a low maximal heart rate Absolute maximal oxygen uptake and also its relation to body weight were both low when compared to normal children of same age The altered association of $V_{O_2 max}$ to heart volume indicates that working capacity measured as aerobic power decrease independently and out of proportion of the circulatory dimensions The main limiting factor should then be the reduced muscle mass and possibly the low maximal heart rate AN undoubtedly has a debilitating effect on the patient and this may partly account for the reduced aerobic performance during later and advanced stages of the disease

ACKNOWLEDGEMENT

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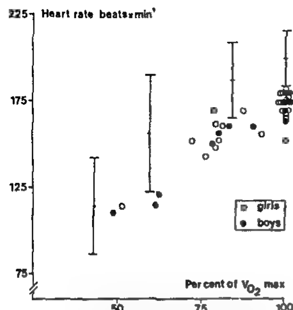


Fig 3 Heart rate and fraction of measured maximal oxygen uptake in 21 AN children For comparison corresponding mean values ±2 S D of 13–14 year old boys are given (12)

and 1 38 l×min⁻¹ in the boys (Table 3) In terms of body weight this represented a VO_2 of 32 ml×kg⁻¹×min⁻¹ and 35 ml×kg⁻¹×min⁻¹ These values are outside the lower range of values found in healthy children of the same age (25) The regression line relating $VO_{2\,max}$ to heart volume was displaced below the expected relationship (14 26) The maximal heart rate was 174 beats×min⁻¹ in the girls and 175 beats×min⁻¹ in the boys The respective maximal lactate values were 11 8 and 12 6 mmol×l⁻¹ and systolic blood pressure averaged 145 and 149 mmHg respectively During exercise HR for a given relative VO_2 was lowered (Fig 3) The normal relationship between cardiac output and VO_2 demonstrated in Fig 4 for 6 patients indicates that the AN children have a normokinetic central circulation when compared with healthy young boys (12) The calculated stroke volumes were well maintained during exercise (Fig 5)

DISCUSSION

The higher proportion of boys in this study than the usual reported incidence of about 10% (4, 5) is partly due to a more active selection of boys

The patients in the present study have low heart rate and subnormal blood pressure at rest These findings are characteristic for long standing starvation (19) and together with the low oxygen consumption at rest they are all probable phenomena of the adaptation to a low metabolic rate

Heart and blood volume were found to be normally related to body weight Hence the decrease in the dimensions of the cardiovascular system seems to be proportional and secondary adapted to the body weight

During exercise the heart rate increased linearly but the maximal heart rate was significantly lower than normal Some caution is necessary when interpreting these findings since it was impossible to apply generally accepted levelling off criteria for maximal work in these patients However *R* values of about 1 1, ventilatory equivalent of around 40 and the high blood lactate values following the work test support the view that the patients were at or near their maximal performance Further studies in AN children before and after rehabilitation with weight gain showed an increase of the maximal heart rate to a normal level (15) From Fig 3 it appears that also at comparable relative loads the heart rate was lower than in healthy boys (12) but this reduc

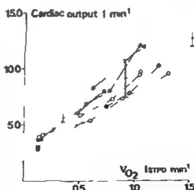


Fig 4 Cardiac output in relation to oxygen uptake at rest supine and during upright exercise in 6 AN patients Corresponding mean values ± 2 S D of 13–14 year old boys are included (12) refer to resting conditions Symbols as in Fig 1

Acta Pædiatr Scand 67 17–24 1978

HAEMOPHILUS INFLUENZAE MENINGITIS

A Comparison between Chloramphenicol and Ampicillin Therapy with Special Reference to Impaired Hearing

M KOSKINIEMI O PETTAY M RAIVIO and S SARNA

From the Department of Neurology and the Children s Hospital University of Helsinki the Hard of Hearing Association and Department of the Public Health Science University of Helsinki Finland

ABSTRACT Koskiniemi M Pettay O Raivio M and Sarna S (Department of Neurology and the Children s Hospital University of Helsinki the Hard-of Hearing Association and Department of the Public Health Science University of Helsinki Finland) Haemophilus influenzae meningitis A comparison between chloramphenicol and ampicillin therapy with special reference to impaired hearing Acta Paediatr Scand 67 17 1978 —131 patients suffering from meningitis due to *Haemophilus influenzae* or *parainfluenzae* were re-examined after 1–15 years using hospital records questionnaires and audiological examination especially to compare chloramphenicol and ampicillin therapy Mortality was 3 8% Subdural effusions occurred in 14 5% of cases uni or bilaterally There was deafness in 2 3% and moderate hearing loss in 8 4% Convulsions appeared later in 6 9% The final otucome was good in 60% The most important factors in prognosis seemed to be the severity of the symptoms and the condition of the patient on admission to hospital No clear difference was seen between the results of chloramphenicol and ampicillin therapy but total loss of vestibular function was found in 3 cases in the ampicillin group and in none in the chloramphenicol group In mortality and deafness the differences in outcome were similar although not statistically significant As these observations show the therapy used in *Haemophilus influenzae* meningitis needs re-evaluation

KEY WORDS Haemophilus influenzae meningitis ampicillin therapy chloramphenicol therapy loss of hearing

Haemophilus influenzae meningitis has conventionally been treated with chloramphenicol initially combined with sulpha and penicillin In 1960 ampicillin therapy gained ground strongly alongside this triple therapy as it is called because in many large series it appeared equally effective alone and was simple to use and atoxic In some cases the fever seemed to continue longer but this was regarded as unimportant (5 18 21) In addition relapses after ampicillin therapy were reported and aroused suspicions about ampicillin resistance (3 8 29) but raising the ampicillin doses to 400 mg/kg weight seemed to help More evidence about true resistance to ampicillin has accrued during the last few years (1 7 9 10 11 26 27) Therefore ampicillin therapy for *Haemophilus influenzae* meningitis has been reevaluated (12) especially in areas where resistance has been reported Gamstorp s report in 1974 (6) about possible hearing loss after ampicillin therapy prompted us to review data on *Haemophilus* meningitis patients and compare the immediate and late results in the different therapy groups especially with regard to impaired hearing

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HAEMOPHILUS INFLUENZAE MENINGITIS

A Comparison between Chloramphenicol and Ampicillin Therapy with Special Reference to Impaired Hearing

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From the Department of Neurology and the Children's Hospital, University of Helsinki, the Hard of Hearing Association, and Department of the Public Health Science, University of Helsinki, Finland

ABSTRACT. Koskiniemi, M., Pettay, O., Raivio, M. and Sarna, S. (Department of Neurology and the Children's Hospital, University of Helsinki, the Hard-of-Hearing Association and Department of the Public Health Science, University of Helsinki, Finland). Haemophilus influenzae meningitis. A comparison between chloramphenicol and ampicillin therapy with special reference to impaired hearing. Acta Paediatr Scand, 67: 17, 1978.—131 patients suffering from meningitis due to *Haemophilus influenzae or parainfluenzae* were re-examined after 1–15 years using hospital records, questionnaires and audiological examination, especially to compare chloramphenicol and ampicillin therapy. Mortality was 3.8%. Subdural effusions occurred in 14.5% of cases uni- or bilaterally. There was deafness in 2.3% and moderate hearing loss in 8.4%. Convulsions appeared later in 6.9%. The final outcome was good in 60%. The most important factors in prognosis seemed to be the severity of the symptoms and the condition of the patient on admission to hospital. No clear difference was seen between the results of chloramphenicol and ampicillin therapy, but total loss of vestibular function was found in 3 cases in the ampicillin group and in none in the chloramphenicol group. In mortality and deafness the differences in outcome were similar, although not statistically significant. As these observations show, the therapy used in *Haemophilus influenzae* meningitis needs re-evaluation.

KEY WORDS: Haemophilus influenzae meningitis, ampicillin therapy, chloramphenicol therapy, loss of hearing

Haemophilus influenzae meningitis has conventionally been treated with chloramphenicol, initially combined with sulpha and penicillin. In 1960 ampicillin therapy gained ground strongly alongside this triple therapy, as it is called, because in many large series it appeared equally effective alone and was simple to use and atoxic. In some cases the fever seemed to continue longer, but this was regarded as unimportant (5, 18, 21). In addition, relapses after ampicillin therapy were reported and aroused suspicions about ampicillin resistance (3, 8, 29), but raising the ampicillin doses to 400 mg/kg weight seemed to help. More evidence about true resistance to ampicillin has accrued during the last few years (1, 7, 8, 10, 11, 26, 27). Therefore ampicillin therapy for *Haemophilus influenzae* meningitis has been reevaluated (12), especially in areas where resistance has been reported. Gamstorp's report in 1974 (6) about possible hearing loss after ampicillin therapy prompted us to review data on *Haemophilus* meningitis patients and compare the immediate and late results in the different therapy groups, especially with regard to impaired hearing.

PATIENTS

We followed up all patients treated for *Haemophilus influenzae* meningitis at the Children s Hospital Univer sity of Helsinki from 1960 to 1974 altogether 131 chil dren The series did not include those remitted from other hospitals because of complications after initial therapy The diagnostic criterion was a positive cerebrospinal fluid (CSF) or blood culture *Haemophilus influen ae* was cultured in CSF in 128 and parainfluenzae in 2 cases In 1 the CSF culture was negative but the stain positive as was also the blood culture which was positive in over 50% of cases altogether The typing of the bacterium and the differentiation of *parainfluen ae* has only been reliable since 1973 (17) and therefore we did not omit the *para influenzae* cases Evidently most *H influenzae* was of type B

In 1960–66 the usual treatment consisted of chloram phenicol (51 patients) dose 100 mg/kg weight 10±4 5 days parenterally 16±6 days altogether In 39 cases it was combined with sulpha and penicillin triple therapy In 1967–74 the usual treatment was ampicillin (59 pa tients) dose 200 mg/kg weight 10±4 days parenterally 16±6 days altogether In 18 cases treatment was started with ampicillin but changed to chloramphenicol because of verified or suspected oversensitivity resistance of the bacterium doubtful effect or appearance of complica tions In 41 cases the initial therapy was trihydrous ampicillin but was changed in 15 cases Anhydrous am picillin was changed in 3 cases out of 28

The sensitivity of the bacterium to chloramphenicol was tested in 96 cases 5 of them showed some resistance Sensitivity to ampicillin was tested in 65 cases a third of them showed some resistance Twenty four patients were treated with cortisone some experimentally for up to 10 days 79% for 4 days or less Sixteen patients in addition received streptomycin for 1 to 20 days mean 6 days Before the appropriate treatment some antibiotic usually penicillin had been given to 34 patients (25 9%) 14 of them for less than 24 hours

There were 67 boys and 64 girls Three of the chil dren were chronically ill 2 had congenital heart fail ure and one Down s syndrome The mean follow up time was 7 years (range 1 to 15 years)

METHODS

Hospital records were perused for information on social background symptoms laboratory findings therapy and outcome A questionnaire was sent to parents of 121 patients The response rate was 113 93 4%

The audiological examination was carried out at least a year after the disease on 101 patients i e 81% of 125 surviving patients The examination included subjective hearing loss otoscopy and audiometry (for details see ref 15) Vestibular function was examined in patients with abnormalities of cochlear function

Standard statistical analyses were carried out For significance testing Student s *t* test and the *chi square* test were used *Multiple analysis* was used to compare the groups of patients in regard to complications especi ally hearing loss convulsions and deaths

Table 1 *Distribution of birth order in sibship for patients and population (24)*

Order in sibship	Patients		Population		
	N	%	*N*	%	*t* test
Only or eldest	33	25 2	677 035	49 4	$p<0\ 01$
Middle	11	8 4	303 930	22 2	n s
Youngest or younger of two	81	61 8	389 386	28 4	$p<0\ 001$
Unknown	6	4 6	–	–	

n s = $p>0\ 05$

RESULTS

Epidemiological aspects

The majority of the patients were from southern Finland as are most of the patients of the Children s Hospital Eighty one patients (61 8%) were the youngest sibs in the family (Table 1) The social background of the par ents was representative of the population (Table 2)

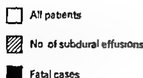
Fig 1 shows the distribution of the new *Haemophilus influenzae* cases in the study pe riod 1960–1974 The annual frequency ranged from 6 to 14, and there was no systematic change

Clinical notes

The age distribution is shown in Table 3 105 patients (80 2%) were ≤3 years old The mean age was 2 0 years and the median 1 6 years The oldest patient was 9 years old and the youngest 2 months 20 days In most cases the symptoms had appeared in the course of a few

Table 2 *Distribution of patients according to the social class of their parents and the cor responding distribution in the population (16)*

Social class	Patients		Population		
	N	%	*N*	%	*t* test
I	21	16 0	139	10 8	n s
II	86	65 6	759	58 7	n s
III	20	15 3	395	30 5	n s
Unknown	4	3 1	–	–	
Total	131	100 0	1 293	100 0	



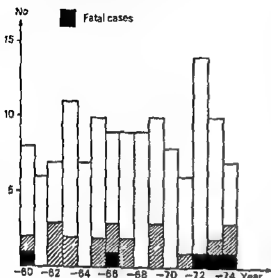


Fig 1 Number of patients annually Fatal cases and number of subdural effusions are included

hours with fever vomiting convulsions and / or unconsciousness Thirty six children were admitted *to hospital* within 24 hours In 84 cases (64 1%) the duration of the symptoms was 3 days or less In 11 (8 4%) the symptoms were diffuse and lasted 14 days *or more* On admission 19 children (14 5%) *were* described as unconscious or reacting poorly one later died In addition 10 children lost consciousness when in hospital four of these died In half the cases the unconsciousness lasted less than 24 hours mean 2 3 days In ? cases the unconsciousness lasted 7 days or more (fatal cases are not included) Besides *poor condition* high fever lowered conscious ness and vomiting initial symptoms included diarrhoea convulsions and divided respiration A few patients had pareses or visual disturbances In 1 case diabetes mellitus was suspected In some cases initial care was focused on the diarrhoea or convulsions and the meningeal irritation at first passed unnoticed On the other hand 1 child was at hospital because of meningeal symptoms despite clear CSF in which no alterations were seen until the third day

Table 3 *Age distribution of the patients*

Age in years	No of patients	[illegible]	Cumulative	
			N	%
Under 0 31	3	2 3	3	2 3
0 31–0 50	14	10 7	17	13 0
[illegible] 51–1 00	25	19 1	42	32 1
1 01–? 00	43	32 8	85	64 9
2 01–3 00	20	15 3	105	[illegible] 2
3 01–4 00	10	7 6	115	87 8
4 01–5 00	5	3 8	1 0	91 6
5 01–6 00	4	3 1	124	94 7
6 01–7 00	1	0 8	125	95 4
7 01–8 00	4	3 1	1?9	98 5
Over 8 00	2	1 5	131	100 0
Total	131	100 1		

Immediate results

In 5 cases (3 8%) the outcome was fatal in the course of 12 hours to 7 days (Fig 1) One of these children was unconscious on admission to hospital the others were tired and in poor condition but conscious The turning point was 12 hours after admission In all fatal cases the symptoms were fulminant under 2 days The ages of these patients were 1 year 4 months 1 year 8 months 2 years 9 months 2 years 10 months and 9 years The oldest was a girl the others boys Bacteriologically the girl had *parainfluenzae* the others *influenzae*

Seven children had cranial nerve symptoms

Table 4 *Appearance of subdural effusions and duration of unconsciousness in days (fatal cases not included)*

Subdural effusions	Duration of unconsciousness (days)			Total
	[illegible]	1	2	
None	91	1?	4	107
Unilateral	9	0	4	13
Bilateral	?	1	3	[illegible]
Total	10?	13	11	1?6

$\chi^2=25\ 2$ $p<0\ 001$ (1st and 2nd [illegible]olumns and ?nd and 3rd rows combined)

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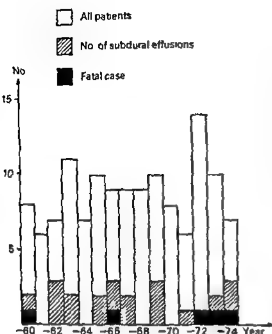


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In 5 cases (3 8%) the outcome was fatal in the course of 12 hours to 7 days (Fig 1) One of these children was unconscious on admission to hospital the others were tired and in poor condition but conscious The turning point was 12 hours after admission In all fatal cases the symptoms were fulminant under 2 days The ages of these patients were 1 year 4 months 1 year 8 months 2 years 9 months 2 years 10 months and 9 years The oldest was a girl the others boys Bacteriologically the girl had *parainfluenzae* the others *influenzae*

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Table 4 *Appearance of subdural effusions and duration of unconsciousness in days (fatal cases not included)*

Subdural effusions	Duration of unconsciousness (days) 0	1	[illegible]	Total
None	91	1[illegible]	4	107
Unilateral	9	[illegible]	4	13
Bilateral	2	1	3	6
Total	102	13	11	126

χ^2_1=[illegible]5 [illegible] $p<0\ 001$ (1st and 2nd columns and 2nd and 3rd rows combined)

Table 5 *Distribution of subdural effusions by age*

Subdural effusion	Age (years) 1 00	1 01–2 00	2 00	Total
None	26	40	46	112
Unilateral	10	3	0	13
Bilateral	6	0	0	6
Total	42	43	46	131

These subsided during the hospital stay Two children showed generalized spasticity and 1 weakness of the shoulder girdle Disturbances of vision occurred in 1 Nineteen children (14 5%) had subdural effusions uni (13) or bi laterally (6) Subdural effusions occurred especially in those children who were unconscious for longer and in the youngest age group (Tables 4 and 5) When unconsciousness lasted more than 2 days, there was usually subdural effusion (in 7 out of 11 cases) the difference is highly significant ($p<0\ 001$) The frequency of subdural effusion was far lower in children over 1 year of age the difference being highly significant ($p<0\ 001$) All bilateral effusions appeared in children under 1 year old None of the patients over 2 years of age had subdural effusions

On leaving hospital 94 children (71 8%) were symptomfree At the conventional follow up after 2 weeks hearing was tested in 40 cases In 11 of these (27 5%) hearing was found to be impaired and in 4 others impairment was suspected altogether 37 5%

Table 6 *Final outcome in the different therapy groups*

Final outcome	Initial therapy (first 3 days) Triple N=39 Amp N=70		Cases	Principal therapy Chlor N=51 Amp N=59		Cases	All patients (N=131) Cases	%
1 Death	Triple	2 6%	1	Chlor	2 0%	1	5	3 8
	Amp	5 7%	4	Amp	6 8%	4		
2 Deafness	Triple	0 0%	0	Chlor	0 0%	0	3	2 3
	Amp	4 3%	3	Amp	3 4%	2		
3 Moderate hearing loss	Triple	15 4%	6	Chlor	11 8%	6	11	8 4
	Amp	7 1%	5	Amp	5 1%	3		
4 Minimal hearing loss	Triple	17 9%	7	Chlor	12 0%	6	15	11 5
	Amp	11 4%	8	Amp	11 9%	7		
5 Impairement of vestib function	Triple	0 0%	0	Chlor	0 0%	0	6	4 6
	Amp	8 6%	6	Amp	6 8%	4		
6 Later convulsions	Triple	7 7%	3	Chlor	7 8%	4	9	6 9
	Amp	7 1%	5	Amp	8 5%	5		
7 1+2+6	Triple	10 3%	4	Chlor	9 8%	5	17	13 0
	Amp	17 1%	12	Amp	18 6%	11		
8 2+3+6	Triple	23 1%	9	Chlor	19 6%	10	23	17 6
	Amp	18 6%	13	Amp	15 3%	9		

Table 7 *Comparability of the groups*

Variable	Initial therapy		Principal therapy		All patients
	Triple $N=39$ Mean ± S D	Ampic $N=70$ Mean ± S D	Chlor $N=51$ Mean ± S D	Ampic $N=59$ Mean ± S D	$N=131$ Mean ± S D
Age of the patient in years	1 8±1 8	2 2±1 9	1 9±1 8	1 9±1 5	2 0±1 8
Social class of the parents 1 ? 3 (16)	2 1±0 5	1 9±0 6	2 1±0 6	1 9±0 6	2 0±0 6
Duration of symptoms before appropriate therapy (days)	5 1±5 6	4 5±4 5	4 5±5 1	4 4±4 5	4 3±4 6
Condition on admission 1=good 2=tired high fever 3=unconscious reacting poorly	2 1±0 5	2 1±0 4	2 0±0 5	2 1±0 4	2 1±0 4
Convulsions or startlings during the first days no of patients	15=38 5%	18=25 7%	18=35 3%	18=30 5%	40=30 5%
Unconscious on admission no of patients	6=15 4%	10=14 3%	7=13 7%	8=13 6%	19=14 5%
Duration of unconsciousness (fatal cases not included)	0 4±1 0	0 4±1 1	0 5±1 3	0 4±1 2	0 4±1 2
Streptomycin treatment no of patients	6=15 4%	2=2 9%	12=23 5%	1=1 7%	16=12 2%
Cortisone treatment no of patients	18=46 2%	4=5 9%	19=37 3%	4=6 8%	24=18 3%
Subdural effusion no of patients	7=17 9%	8=11 4%	7=13 7%	7=11 9%	19=14 5%

Death occurred in 1 patient (2 0%) treated principally with chloramphenicol and 4 (6 8%) treated with ampicillin (Table 6) The frequency of subdural effusions varied from 11 4 to 17 9% The differences between the different therapy groups are not statistically significant The hearing of all the patients treated with chloramphenicol remained normal during the hospital stay At follow up after 2 weeks impairment was noted or suspected in 6 (11 8%) and in the ampicillin patients in 9 (15 2%) The comparability of the groups is seen in Table 7 Clear differences are seen in the use of streptomycin and cortisone but the symptoms and the social background are the same

Follow *up results*

The final outcome as judged from the questionnaire and audiological examination was good in 75 cases (57 3%) Eighteen children (13 7%) have to be followed up at regular intervals 1 was institutionalized One child with congenital heart failure died more than 1 year after the meningitis Ninety five children are able to live normally without special aids

During the follow up period 80 children were of school age of these 71 (88 7%) had marks that were average or better and 9 (11 3%) marks below the average

Three children had uni or bilateral deafness (Table 6) 11 (8 4%) a moderate defect and 15 a minimal defect that was not handicapping The duration of unconsciousness showed no association with the hearing defects Neither subdural effusions nor streptomycin or cortisone treatment showed any association with loss of hearing All 6 cases of defective vestibular function were found in the groups initially treated with ampicillin

Eight children had grand mal attacks and 1 absences (6 9%) The appearance of convulsions in later life were associated with fulminant symptoms ($p<0\ 001$) poor general condition on admission to hospital ($p<0\ 05$) prolonged unconsciousness ($p<0\ 001$) subdural effusions ($p<0\ 001$) and young age ($p<0\ 001$ all under 1 5 years old)

Two factors at most a short period of unconsciousness and the profession of the father had some association ($p<0\ 05$) with a good outcome The following factors had no relation to the final outcome sex antibiotic

Table 8 *Variables most clearly differentiating the groups with good and poor final outcome*
The correlations between discriminant function and variables Variables with correlations above 0 300 are shown

Variables	Good final outcome (based on questionnaire and audiological examination)	Poor final outcome (died deaf epileptic)
1 Duration of unconsciousness (days)		0 782
2 Subdural effusions (0=none 1=unilateral 2=bilateral)		0 477
3 Duration of parenteral therapy (intravenous+intramuscular) days		0 415
4 Condition on admission to hospital (1=good 2=tired high fever 3=unconscious)		0 378
5 Antibiotics before admission to hospital (days)	−0 308	

treatment at home; streptomycin and cortisone treatment

The clearest difference associated with the initial treatment was found in impairment of vestibular function No cases were found in the group that initially received the triple treatment, whereas six cases occurred in the group initially treated with ampicillin The same was true of deafness The group initially treated with ampicillin included 3 deaf children the chloramphenicol group none In regard to moderate hearing defects the relation was the reverse 6 (15 4%) and 5 (7 1%), but in fact these results are not directly comparable

Comparison of the irreversible and severe defects (death, deafness convulsions) shows that ampicillin as initial treatment was less favourable The difference was *not statistically significant* There was no difference between the two therapy groups with respect to general condition or symptoms

When the cases with a good outcome were compared with the deaths and cases of deafness and epilepsy (Table 8) *multiple discriminant analysis* involving 16 variables described the latter group as follows no antibiotics at home condition poor on admission to hospital unconsciousness, long parenteral treatment and subdural effusions The final outcome was good when antibiotics were given at home before admission to hospital, the condition was good on admission conscious ness was maintained at the time, there were no subdural effusions and parenteral treatment was short i e the illness ran a mild course

DISCUSSION

Sequelae were as numerous in this study as in previous reports but the distribution was different Mortality, 3 8% was low compared with other series, and the number of patients without handicapping sequelae, near 60%, was about average (2 13 14 18 23)

Subdural effusions were noted in 14 5%, all under 2 years old and later convulsive disorders in 6 9% These figures resemble the few mentioned in earlier reports (18, 20 21) The frequency of impaired hearing was surprisingly high Cases of deafness and moderate hearing loss were much more frequent than expected Hearing was impaired in over 20% In the earlier reports the usual figure was about 10% (20 25) but these reports mostly referred to bacterial meningitis in general The illness clearly affected the youngest members of the family Turk reported the same finding in 1975 (28)

The defects found bore no clear relationship to the medication Deaths and deafness were more frequent in the ampicillin than in the triple therapy group but the difference was not significant All cases of vestibular

defects occurred in the ampicillin group From this it could be argued that ampicillin is of less value than chloramphenicol in protecting the inner ear against damage by Haemophilus influenzae On the other hand we do not know how far the inner ear damage is repaired in the course of time In this respect chloram phenicol was in a more favourable position because the follow up time in that group was longer But vestibular function once lost does not return In the ampicillin therapy group vestibular function was lost in 3 but in the chloramphenicol group in none Medication was changed in 18 members of the group started on ampicillin therapy Chloramphenicol may be more trustworthy however and it has always had it s supporters (19) None of the feared side effects of chloramphenicol was seen and we found no mention of them in reports on the results of *H influenzae* therapy Changes from ampicillin tended to be made especially in the group started on the trihydrous derivative in 15 out of 41 and anhydrous ampicillin in 3 out of 28 The effect of the anhydrous derivative on the final out come could not be seen because the groups were too small The outcome was not im paired by antibiotics given before admission to hospital (4)

It is worth noticing that the conventionally measured resistance of the bacterium bore no relationship to the final outcome This links up with one of the hazards in evaluating our re sults i e the ampicillin dose used (200 mg/ kg weight) may have been too low Unfor tunately the length of intravenous therapy could not be ascertained in all cases How ever Fig 1 shows that something is happen ing Nowadays one death is seen every year We may suspect that the illness is becoming more severe but our groups did not differ in clinical aspects and both basic care and in tensive care have been the same in the six ties and seventies at the Children s Hospital The frequency of the illness is about the same too Earlier reports are contradictory (14 22) Altogether confidence in ampicillin therapy in *Haemophilus influenzae* meningitis is with reason changing to caution and needs further re evaluation

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SEASONAL VARIATIONS IN WEIGHT OF CHILDREN ATTENDING AN UNDER FIVES CLINIC IN LESOTHO

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ABSTRACT Cohen N and Clayden A D (Department of Community Health University of Nottingham Medical School Nottingham England) Seasonal variations in weight of children attending an under fives clinic in Lesotho Acta Paediatr Scand 67 25 1978 —The incidence of weight loss between successive visits prevalence of under centile 3 weight for age and over centile 97 showed a marked bimodal annual variation for 1 243 children attending an under fives clinic 9 949 weighings on 661 boys and 682 girls over a five year period contributed to the database Season of birth also significantly influenced the centile distributions of weight attained for age Children over 1½ years old showed considerably higher rates of weight loss (about 1 in 5 children attending in their fifth year of life) than children younger than the mean age of weaning The rates of weight loss together with seasonal variation was considerably higher for a group of Regular Attenders to the clinic than for a group of Low Attenders Seasonal influences on growth need to be taken into accounts in evaluating the quality of care and outcome produced by under fives clinics

KEY WORDS Seasonal variation weight growth under fives clinics Lesotho

Seasonal fluctuation in incidence is a general principle of epidemiology observed in the study of many phenomena and is important evidence of an environmental factor in aetiology Seasonal variations in growth rate have been reported in studies of groups of well fed American British and Japanese children (6 7 9 12) In subsistence societies more marked seasonal effects on growth morbidity and mortality in childhood are recognised (4) The growth of such children follows cycles of impairment failure and recovery which have probably been the norm throughout evolution Approaches to the care and assessment of these children therefore need to take into account the likelihood of considerable periodic variation in their potential for maturation and development as well as their capacity to resist infection Where seasonal variations in the tempo of growth are recorded their analysis should provide feedback of the effects of determining environmental factors as well as an indication of the effectiveness of any interventions proposed If seasonal variation is overlooked as a cause of variation in growth and morbidity its magnitude may be so great as to alter the significance of the results obtained (1)

Children attending under fives clinics are weighed regularly and the weight records used as an indication of the well being and state of nutrition of the individual child But since the data generated are considerable the implications of trends effecting larger groups of children may be missed Weight for age is the most used single measurement for assessment

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of nutritional status recommended by W H O Although, particularly in regions where the date of birth is not officially recorded it is accepted that weight for age alone has many drawbacks as a means of assessing the growth and development of young children (11)

This report which forms part of a five year semi longitudinal follow up examines seasonal variations in some measures of the weight of village children in Lesotho attending an under fives clinic A simple partially age independent method of surveillance for seasonal effects on growth has been tested namely, monitoring of the incidence of weight loss between successive measurements

METHODOLOGY

The data relates to 1243 children who attended an under fives clinic at St James Hospital Mantsonyane Lesotho between October 1969 and August 1974 9949 weighings on 661 boys and 682 girls contribute to the database Mothers were encouraged to bring their children on a monthly basis But in practice such regularity of attendance was rarely achieved

Population

Children attending the clinic were almost entirely Basotho (Southern Sotho) there were a few Xhosa Zulu and Pedi Basotho in the catchment area live in scattered villages grouping not more than 40–50 families dependent on a subsistence agricultural economy The size of the population under five years old accessible to the clinic was estimated to be of the order of 2000 Lesotho is one of the poorer countries in the world with average family earnings below 100 U S dollars

Geography and climate

The villages in which the children lived are in the mountainous eastern part of Lesotho at an altitude of 7000–9000 feet The climate in the mountains is severe with dry cold winters (sometimes including snow) and heavy summer rains Seasonal differences in climate are therefore marked Annual variations are also frequent with cycles of failure of the rains leading to drought or unusually wet summers washing away the crops The arable land has been seriously over grazed and as well as the steep terrain considerable areas of erosion restrict cultivation of the staple cereal crops—wheat maize and millet

Seasons

Four seasons have been defined December–February (corresponding to summer) March–May June–August (corresponding to winter) and September–November Crops are planted after the first rains about September–October harvesting takes place from the early months of the year

Feeding water supply and sanitation

Breast feeding was usual with weaning onto sour milk (mafi) or a watery porridge Increasing numbers of children were also offered bottles containing dilute mixtures of commercially prepared powdered milk Many mothers gave small quantities of cow s milk to their children The staple cereal for older children was maize (pappa) together with millet often made into a gruel (lesheleshele) Steamed bread was prepared from whole wheat Vegetable protein from peas beans and wild vegetables (meroho) was supplemented occasionally by meat Boys but not girls were given eggs

All drinking water came from generally unprotected springs or streams There was no piped water supply in any of the villages In summer older children would bathe in the streams but otherwise washing the whole body was rare Pit latrines were not used throughout the region during the time of the study

Weight measurements records and analysis

Children were weighed without clothes on a transverse beam balance by mothers trained and supervised by the clinic nurse Dates of birth were as given by the mothers since there was no official record (Maternal basic literacy was estimated to be over 80% and the ages of the children are thought to be more reliable than is usual for most rural communities) Clinic visits were registered by month and the calculation of age at each visit is based on mid month figures In addition to the Road to-Health chart taken home by the mother details were entered in the clinic register for each child of name sex village date of birth immunisation status dates and weights at clinic visits After transfer and coding of the raw data the necessary conversions calculations and printout were programmed for the Nottingham University ICL 1906A computer

Expression of results

Weight loss between successive visits is defined as any decrease in weight following a weighing on the previous visit even if more than a month had elapsed between measurements In order to standardise for the obvious bias produced by seasonal variations in attendance findings are reported in terms of rates per 1000 visits of children weighed at the clinic Male and female data have been sometimes grouped to condense the presentation Regular Attenders are defined as children who have attended the clinic at least ten times over any period of two years Low Attenders have visited less frequently Centile comparisons of weight for age measurements refer to cross sectional type weight reference values published for the same population (2)

RESULTS

Season of birth and weight attained for age

Centile distributions of weight attained for age have been produced for children boys and girls separately born in the four seasons of the

Table 1 *50 centile (median) of weight attained for age of boys and girls born in March–May June–August September–November December–February*

	Birth month							
	Male				Female			
Age group	3 4 5	6 7 8	9 10 11	12 1 2	3 4 5	6 7 8	9 10 11	12 1 2
0 08–0 24	5 0	5 5	4 7	5 5	4 5	4 7	4 8	4 5
0 25–0 49	6 0	5 9	5 8	6 4	5 7	5 9	6 0	6 0
0 50–0 74	6 7	6 9	6 9	7 2	6 5	6 5	7 0	7 1
0 75–0 99	7 6	8 0	7 7	7 7	7 3	7 9	7 9	7 5
1 0–1 24	8 3	8 5	8 5	8 6	7 9	8 3	8 3	7 9
1 25–1 49	8 7	8 8	9 4	9 6	8 6	8 7	8 9	8 7
1 50–1 74	9 1	9 4	10 0	9 9	8 8	9 3	9 5	9 2
1 75–1 99	10 0	9 9	10 4	10 2	9 5	9 5	10 0	9 5
2 0–2 24	10 7	10 9	10 6	10 6	10 0	10 5	10 4	10 0
2 25–2 49	10 6	11 1	11 3	11 3	10 2	10 9	10 9	10 6
2 50–2 74	10 9	11 3	11 8	11 8	10 9	11 3	11 7	10 9
2 75–2 99	11 3	12 1	12 7	11 8	11 1	12 2	12 3	11 4
3 0–3 49	12 4	12 3	13 0	13 2	12 4	12 3	12 7	12 3
3 50–3 99	13 2	13 6	13 6	13 7	13 2	13 2	14 1	13 6
4 0–4 49	15 0	14 5	13 6	14 7	13 6	14 5	14 1	14 1
4 5–4 99	14 1	15 4	15 0	–	15 0	14 8	15 0	14 5

year For boys and girls the median (50 centile) for weight of March–May births tended to be the lowest from 6 *months old (10 out of* 14 age groups for boys and 12 out of 14 for girls) (Table 1) Using Friedman s 2 way analysis of variance the *hypothesis that there* was no association between season of birth and weight for age was rejected at the 99 9% level The extreme centiles 3 and 97 showed no consistent seasonal trends in either sex

Month of visit and incidence of weight loss

When analysed by month of visit the incidence of weight loss between successive visits showed a marked bimodal annual variation (Fig 1) The lowest rates of weight loss between visits were in January 97 3 per 1000 and June 99 4 per 1000 visits The rates were higher for children visiting the clinic in March 168 7 *per 1000* visits and were again high in the period August–November reaching a peak of 188 0 per 1000 visits in September The proportion of *children recorded* as having lost weight from the previous visit was 0 22 in September and November 0 20 in March and a minimum of 0 06 *in* July Put more simply about 1 in 5 of children visiting the clinic from August to November or in March showed weight loss from the previous visit The average *weight loss between visits* varied according to the month of visit between 0 25 kg and 0 85 kg Naturally the younger and lighter *children showed* less absolute weight loss between successive visits

Children whose weight for age falls below *Lesotho centile* 3 are severly undernourished The prevalence of children whose weight fell below centile 3 for their age showed a similar *monthly* variation (Fig 1) In March the weights of 54 2 per 1000 of children attending were under centile 3 the rate falling to 22 1 *per* 1000 *in July* and rising again to 41 7 per 1000 in December Conversely the rate of children attending whose weight for age exceed *centile 97* was highest in June 50 7 per 1000 falling to 21 4 per 1000 in December and 18 1 per 1000 in March It is noteworthy *that frank kwashiorkor* was not observed at all in this series

Age at visit and incidence of weight loss

Fig 2 compares monthly variation *in the* incidence of weight loss between successive visits of *children* over and under 1½ years old

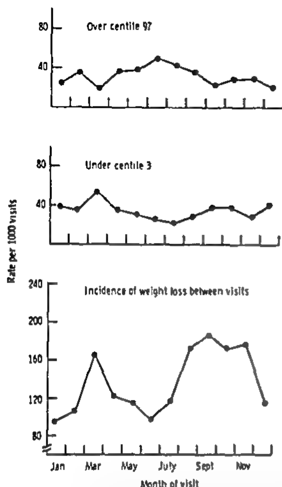


Fig 1 Monthly variation in (*a*) incidence of weight loss between successive visits (*b*) prevalence of under centile 3 weight for age (*c*) prevalence of over centile 97 weight for age

a cut off point chosen as representing the mean age for weaning For both older and younger groups of children it will be seen that the bimodal pattern is similar with considerably higher rates in the group of children over 1½ years old Up to the age of 18 months to two years the incidence of weight loss increased markedly from 42 0 to 169 2 per 1 000 visits About 1 in 5 of children attending in their fifth year of life showed weight loss from the previous visit

Frequency of visits and incidence of weight loss

A bimodal annual distribution of weight loss between successive visits was observed both for Regular Attenders and for Low attenders (Fig 3) However, the rates of weight loss for the group of Regular Attenders were considerably higher than for the group of Low Attenders' both in the March peak 201 1 as compared with 155 5 per 1 000 visits, and in the August–December peak, 160 5–222 2 as compared with 164 5–177 7 per 1 000 visits Although the pattern of seasonal variation was comparable for each month, less of the 'Regular Attenders group were under centile 3 and conversely higher proportions exceeded centile 97

DISCUSSION

Overall rates of weight loss episodes reported would not be unusually high for children in many similar poor communities But the seasonal fluctuations in weight patterns are striking It seems logical that in the absence of suitable conditions for catch up growth these repeated periods of growth faltering are responsible for the fact that after the first few months of life village children in Lesotho are both lighter and gain weight slower than more privileged groups (2)

It is possible to speculate on factors such as temperature and sunshine which produce seasonal variation in the growth of well fed

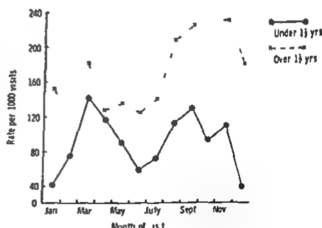


Fig 2 Monthly variation in incidence of weight loss between successive visits for children under 1 5 years and 1 5–4 9 years old

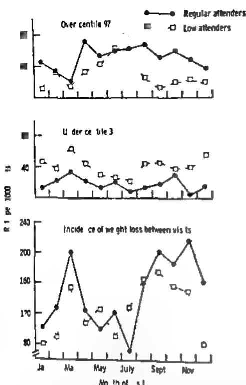


Fig 3 Monthly variation for Regular and Low Attenders in (*a*) incidence of weight loss between successive visits (*b*) prevalence of under centile 3 weight for age (*c*) prevalence of over centile 97 weight for age

children Even where such seasonal differences are small it is not considered satisfactory to assess a child s growth over periods of less than a year (7) *But it is important to* emphasise that the seasonal effects described here involving not only a slowing in weight gain but actual weight loss in so many instances are of a completely different intensity than for children in less disadvantaged situations It is therefore clear that *neglect of* seasonal variation in Lesotho would introduce serious errors into the interpretation of cross sectional or semi longitudinal surveys using weight for age as an indicator for example of nutritional status in childhood or the outcome of attendance at under fives clinics In the Gambia Billewicz (1) calculated that differences equivalent to about one third of the range of weight for age published for African populations at the time could be produced by seasonal fluctuations alone Except for during the first six months of life weight gain as in Lesotho depended not only upon age but season Season of birth also dictated the average pattern of weight gain during early life though the differentials had eased by two years

The biannual peaks in rates of weight loss episodes draw attention to adverse environmental factors which vary seasonally The peak in March at the end of the wet season follows the time of highest incidence of diarrhoeal disease both water related and non water borne such as flies The high rate of weight loss episodes up to the mean time for weaning is probably related to the incidence of this gastro intestinal disturbance which Mata et al (8) in a Guatemalan village showed to be least in the uniquely breastfed period and increasing with the weaning process to reach a maximum at the time of complete weaning The sustained high level of weight loss episodes from August to November coincides with the period of frequent respiratory and other minor infections at the end of winter as well as increasing food shortage Although the diet of the children is marginally adequate in protein throughout the year it is often markedly deficient in calories at this time because either sufficient staple cereal is not available or younger children cannot obtain less bulky supplements

The persistent bimodal seasonal pattern in older children points to the importance of sustained undernutrition in association with continuing susceptibility to disease In contrast the Gambian children of 0–4 years described by McGregor et al (4) only experienced adverse effects on weight height and morbidity during the rainy season which coincided with an increase in malaria and other infections

There are several possible explanations for the apparent consistent tendency to lighter weight for age of March–May births Their mothers certainly face a season of increasing

food shortage which could reduce lactation The peak period for weaning of March–May births in September–November of the following year also coincides with the season of most serious food restrictions so that less solid food may be available for them at this critical stage

It must also be appreciated that these results *relate to selected groups of children* Within each group are further distinct subsets Regular Attenders includes an advantaged subset whose distribution of weight for age is comparatively high throughout the year as well as a subset who lose weight more frequently at certain seasons There is no information given on the variability between individuals in different seasons or the way in which one child s growth may vary from one year to the next It should not be thought either that such marked seasonal variations are an immutable Law of Nature elsewhere in Africa or even in Lesotho In Tanganyika for example well nourished children showed *far less seasonal influence on growth (10)* A marked seasonal pattern to the capacity of the young to thrive is one of the stigmata of poverty and a subsistence economy

Few published studies have shown beneficial effects as a result of attendance at an under fives clinic *(3 5) By recognising the* importance of seasonal variations emphasis is placed on the need for assessment in each situation of those wider environmental factors such as diet sanitation water supply and disease patterns which ultimately determine the extent to which children in rural villages or urban shanty towns can realise their potential for growth and survival Where resources are scarce it is surely most appropriate to focus them on the periods of greatest risk A challenge to epidemiology is to provide simple and sensitive tools which can be used by basic health workers with little training and no special facilities to monitor and evaluate their work Such measures should not be separate from nor complicate their primary responsibility which is to provide both preventive and curative care Calculation of the incidence of weight loss between successive measurements appears to go some way to meet these criteria For the individual child this direct observation already forms the basis for assessment of progress as well as advice to the mother Another advantage is that there is no necessity to know the precise age of the child The computer techniques used for this research have no place in the field where we believe that effective audit can be achieved using single sampling systems This aspect we intend to develop in future presentations

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food shortage which could reduce lactation The peak period for weaning of March–May births in September–November of the following year also coincides with the season of most serious food restrictions so that less solid food may be available for them at this critical stage

It must also be appreciated that these results relate to selected groups of children Within each group are further distinct subsets 'Regular Attenders includes an advantaged subset whose distribution of weight for age is comparatively high throughout the year as well as a subset who lose weight more frequently at certain seasons There is no information given on the variability between individuals in different seasons or the way in which one child s growth may vary from one year to the next It should not be thought either that such marked seasonal variations are an immutable Law of Nature elsewhere in Africa or even in Lesotho In Tanganyika for example well nourished children showed far less seasonal influence on growth (10) A marked seasonal pattern to the capacity of the young to thrive is one of the stigmata of poverty and a subsistence economy

Few published studies have shown beneficial effects as a result of attendance at an under fives clinic (3 5) By recognising the importance of seasonal variations emphasis is placed on the need for assessment in each situation of those wider environmental factors such as diet sanitation, water supply and disease patterns which ultimately determine the extent to which children in rural villages or urban shanty towns can realise their potential for growth and survival Where resources are scarce it is surely most appropriate to focus them on the periods of greatest risk A challenge to epidemiology is to provide simple and sensitive tools which can be used by basic health workers with little training and no special facilities to monitor and evaluate their work Such measures should not be separate from nor complicate their primary responsibility which is to provide both preventive and curative care Calculation of the incidence of weight loss between successive measurements appears to go some way to meet these criteria For the individual child this direct observation already forms the basis for assessment of progress as well as advice to the mother Another advantage is that there is no necessity to know the precise age of the child The computer techniques used for this research have no place in the field where we believe that effective audit can be achieved using single sampling systems This aspect we intend to develop in future presentations

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The Lesotho Ministry of Health and Catholic Relief Services have provided continuing support for the clinic Oxfam contributed to building costs and the salary of a trained worker Results have been achieved by the total commitment of all members of the staff as well as those mothers who undertook weighing Mrs I Summers aided the final analysis of the data

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RELATIVE UNDERWEIGHT IN CYSTIC FIBROSIS AND ITS PROGNOSTIC VALUE

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ABSTRACT Kraemer R Rudeberg A Hadorn B and Rossi E (Department of Paediatrics University of Berne Berne Switzerland) Relative underweight in cystic fibrosis and its prognostic value Acta Paediatr Scand 67 33 1978.—On the basis of observations in 117 children with cystic fibrosis seen from January 1956 to June 1976 it is demonstrated that the relative underweight (weight loss corrected for height) is most pronounced in children with predominantly pulmonary symptoms The degree of underweight closely correlates inversely with survival Because of its prognostic value it is recommended that this clinical parameter be included in the checkups which are periodically carried out on children suffering from cystic fibrosis

KEY WORDS Cystic fibrosis relative underweight prognostic value

The therapy for cystic fibrosis of the pancreas (mucoviscidosis) is based on a multifactorial approach The comparison of the effect of different forms of therapy has been made by comparing survival in the different treatment groups (5 7 8 9 10 13 16 20)

For future similar studies it seems desirable to use criteria which can be applied during the life time of the patient For this purpose different scoring systems which attempt to express clinical severity have been proposed (1 3 14 18) The grading of clinical severity of the disease also seems important from the point of view of management and prediction of long term survival (11) None of the previously published studies mentions relative underweight as a clinical parameter entering into the score

In the present study we were interested to see how useful relative underweight calculated according to the curves of Tanner (19) could be as a prognostic tool and we also investigated its correlation with later survival

PATIENTS AND METHODS

The patients were 117 children with cystic fibrosis seen between January 1956 and June 1976 [1] The patients were divided into 3 groups according to the symptoms presented at the time of diagnosis

Group I Predominantly pulmonary problems with cough rales over both lungs pneumonia but no symptoms from the gastrointestinal tract

Group II Pulmonary and gastrointestinal symptoms frequent cough obvious lung involvement but also abnormal fatty and bulky stools

Group III Only gastrointestinal symptoms at the time of diagnosis No lung involvement

Patients with meconium ileus seen during this period ($N=37$) and patients detected because of a positive family history ($n=15$) were excluded

The relative underweight (weight loss corrected for height according to the curves of Tanner (19) was calculated by the following method as illustrated in Fig 1

We assume that the weight of a boy aged 12 years is 16 kg and his height 126 cm This height corresponds to the 50th percentile of the chronological age of 8 years the corresponding weight is 24 2 kg as illustrated in Fig 1 His relative underweight therefore is 8 2 kg

In each of the three symptomatic groups the relative

[1] A number of physicians assisted us in the care of these patients especially Drs B Frolet E Stoll R Hagmann H Gaze and D Kaiser

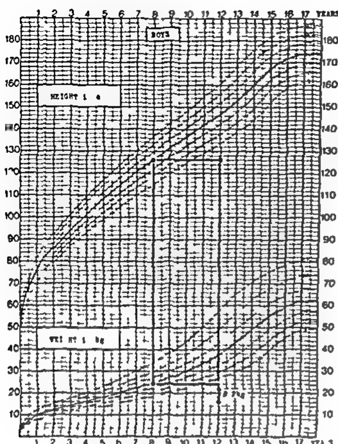


Fig 1 Illustration of the method for expressing relative underweight (weight loss corrected for height) according to the curves of Tanner (*19*)

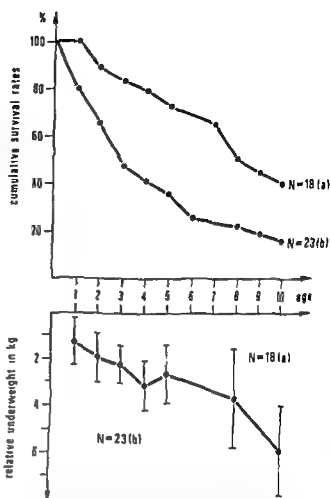


Fig 2 Group I Cumulative survival rates (above) and *relative underweight (below)* in relation to the age in years in 41 patients with pulmonary symptoms at time of diagnosis (*a*) Below mean underweight (*b*) above mean underweight

underweight was calculated at age 1 2 3 4 5 8 and 10 years The patients were then divided into two sub groups If the underweight was below the mean of the whole group the patient was assigned to subgroup *a c* or *e* respectively If on the other hand the underweight was more than the mean of the whole group the pa tient was assigned to subgroup *b d* or *f* respectively Cumulative survival was calculated for each of the six subgroups (*a–f*) by the method of Cutler & Ederer (2)

As a statistical evaluation we chose the method with contingency tables comparing survival and death fre quencies within the symptomatic groups (I–III) classi fied according to three degrees of relative underweight (more than mean +1/2 S D /between the two 1/2 S D / less than mean −1/2 S D)

RESULTS

As the figures 2–4 clearly show the increas ing degree of underweight is most pronounced in the group with pulmonary symptoms at the time of diagnosis (Fig 2) At age 10 for ex ample the relative underweight of group I is more than twice that of the other groups

In addition to this observation the figures show that the patients whose relative under weight is less than the group mean survive longer than those with more underweight than the mean

Table I shows the contingency tables of the three symptomatic groups illustrating the relationship between three degrees of relative underweight (rows) and the criterion living or dead (columns) during the observation time from birth up to age 10 The statistical evaluation shows that in this two way classi fication the null hypothesis of independence can be rejected at a conventional level ($p>0.05$ by one tail) in group *III* and even at a higher level ($0.01>p>0.001$) in the other groups Thus inspection of the observed pro portions reveals that there is a significant cor

Table 1 *Significance levels for comparison of survival and death frequencies in different symptomatic groups of children according to degree of relative underweight*

	Group I respiratory symptoms			Group II respiratory and gastrointestinal symptoms			Group III gastro-intestinal symptoms		
	Alive	Dead	Total	Alive	Dead	Total	Alive	Dead	Total
Underweight									
More than mean+½ S D of the group	5	10	[illegible]	3	5	8	4	4	8
Between the two ½ S D of the group	4	10	14	11	7	18	12	1	13
Less than mean−½ S D of the group	11	1	[illegible]	17	2	19	9	1	10
Total	20	21	41	31	14	45	25	6	31
Statistical evaluation									
3×2 contingency table according to the equation of Brandt Snedecor									
$\chi^2 = \frac{n^2}{x(n-x)}\left(\sum_i^k \frac{x_i^2}{n_i} - \frac{x^2}{n}\right)$	$\chi^2=12\ 1$			$\chi^2=7\ 9$			$\chi^2=6\ 5$		
Significance level	$0\ 01>p>0\ 001$			$0\ 025>p>0\ 01$			$0\ 05>p>0\ 025$		

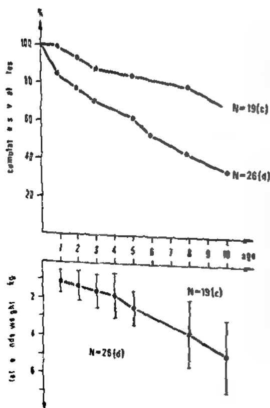


Fig 3 Group II Cumulative survival rates (above) and relative underweight (below) *in relation to the age in* years in 45 patients with pulmonary and gastrointestinal symptoms at time of diagnosis (*c*) Below mean underweight (*d*) above mean underweight

relation between high relative underweight and lethal outcome The significance of this dependency is again most pronounced in the group with pulmonary symptoms at the time of diagnosis

DISCUSSION

The study shows that relative underweight is of prognostic value since a higher degree of underweight correlates closely with poor survival It is interesting that this effect is more clearly expressed in the group with pulmonary symptoms only than in the other groups The question arises as to the reasons for this good inverse relationship between underweight and survival Previous studies by other investigators (3 6 7 15) have demonstrated that gastrointestinal involvement plays

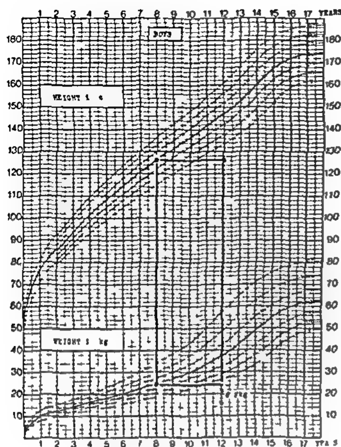


Fig 1 Illustration of the method for expressing relative underweight (weight loss corrected for height) according to the curves of Tanner (19)

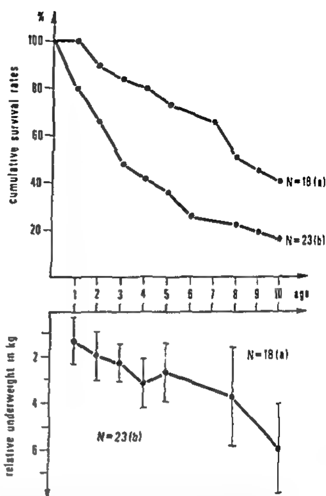


Fig 2 Group I Cumulative survival rates (above) and relative underweight (below) in relation to the age in years in 41 patients with pulmonary symptoms at time of diagnosis (*a*) Below mean underweight (*b*) above mean underweight

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RESULTS

As the figures 2–4 clearly show, the increasing degree of underweight is most pronounced in the group with pulmonary symptoms at the time of diagnosis (Fig 2) At age 10 for example the relative underweight of group I is more than twice that of the other groups

In addition to this observation the figures show that the patients whose relative underweight is less than the group mean survive longer than those with more underweight than the mean

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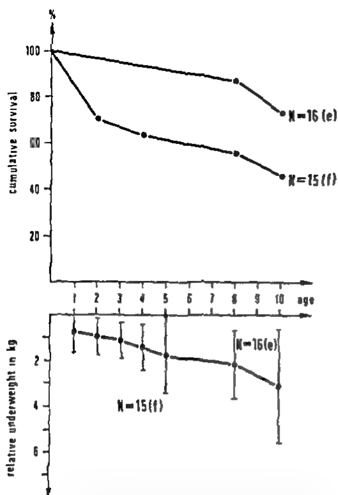


Fig 4 Group III Cumulative survival rates (above) and relative underweight (below) in relation to the age in years in 31 patients with gastrointestinal symptoms only at time of diagnosis (*e*) Below mean underweight (*f*) above mean underweight

a relatively minor role in determining growth and weight gain and later survival It is likely therefore that the common cause of the more pronounced underweight and poorer survival in the subgroups is the higher degree of pulmonary involvement

Finally we suggest that the measurement and calculation of relative underweight should be included in the routine examinations which are carried out on these patients

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THE PLASMA LEVELS OF 25 HYDROXYVITAMIN D IN PATIENTS WITH VARIOUS LIVER DISEASES AND THE RESPONSE OF 25 HYDROXYVITAMIN D TO VITAMIN D TREATMENT

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From the Department of Paediatrics University Hospital Osaka Japan

ABSTRACT Seino Y Shimotsuji T Kai H Ikehara C and Yabuuchi H (Dept of Paediatrics University Hospital Osaka Japan) The plasma levels of 25-hydroxyvitamin D in patients with various liver diseases and the response of 25-hydroxyvitamin D to vitamin D treatment Acta Paediatr Scand 67 39 1978 —The mean plasma levels of 25 hydroxy vitamin D (25-OH D) were measured before and after the administration of 2000 units of daily oral vitamin D_2 for a period of 2 weeks in 9 normal infants and children 7 infants with neonatal hepatitis and persistent neonatal hepatitis and 4 infants with congenital biliary atresia The mean plasma level of 25-OH D increased significantly from 19 5±3 7 (S E) ng/ml to 34 0±6 8 (S E) ng/ml after administration of vitamin D_2 in controls ($p<0\ 05$) The mean plasma level of 25 OH D also increased from 8 0±2 1 (S E) ng/ml to 22 1±2 6 (S E) ng/ml after vitamin D treatment in hepatitis group ($p<0\ 05$) In patients with congenital biliary atresia vitamin D treatment did not affect the plasma levels of 25-OH D

KEY WORDS 25-hydroxyvitamin D neonatal hepatitis congenital biliary atresia

Hepatic osteodystrophy has been recognized in patients with various liver diseases in infancy and childhood (5 10 13) and low concentrations of plasma 25 OH D have been reported in patients with various liver diseases particularly with liver cirrhosis in adults (6 14) The cause of hepatic osteodystrophy may be in part due to lack of vitamin D or disturbance of vitamin D metabolism in the liver The present study was designed to assess plasma 25 OH D concentrations and their responses to vitamin D treatment in patients with various liver diseases during childhood

MATERIALS AND METHODS

Sixteen patients were studied Diagnosis of neonatal hepatitis or persistent neonatal hepatitis was made in 11 patients (4 males and 7 females) aged from 2 weeks to 2 years on the basis of clinical manifestations and liver function tests (1 7 8) The livers of patients with neonatal hepatitis were enlarged and firm and the stool became clay colored after several weeks These patients showed prolonged obstructive jaundice and moderately elevated transaminases which lasted for more than a year in 2 infants

Five patients (1 male and 4 females) aged from 1 month to 5 years were proved by surgery to have congenital biliary atresia An obstructed duct was anastomosed to the duodenum in each case Nine normal infants and children aged from 2 weeks to 5 years were studied as controls All plasma samples were obtained early in the morning after overnight fasting during the winter season

The plasma levels of 25 OH D were measured by the competitive protein binding assay which has previously been reported (12) In 9 normal infants and children 7 cases of hepatitis and 4 cases of congenital biliary atresia the plasma levels of 25-OH D were measured immediately before and after the administration of 2000 units of daily oral vitamin D for a period of 2 weeks

The plasma levels of 25-OH D were also measured immediately before and 48 hours after subcutaneous injections of 20000 units of vitamin D_2 in 7 normal infants

Acta Pædiatr Scand 67 39–42 1978

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The plasma levels of 25-OH D were also measured immediately before and 48 hours after subcutaneous injections of 20000 units of vitamin D_3 in 7 normal infants

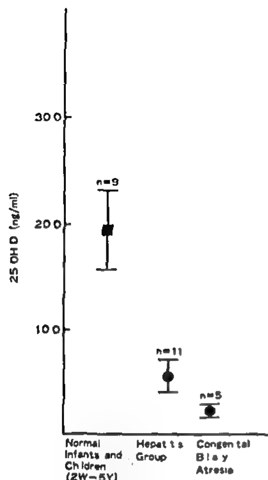


Fig 1 Plasma 25 OH D levels in normal subjects and patients with liver diseases (Hatched area shows mean±1 S E)

and children and in 3 cases of congenital biliary atresia 1 male and 2 females aging from 3 months to 1 year

RESULTS

As shown in Fig 1 the mean plasma level of 25 OH D in controls was 19 5±3 7 (S E) ng/ml (n=9) The mean plasma levels of 25 OH D in patients with hepatitis and congenital biliary atresia were 5 7±1 4 (S E) ng/ml (n=11) and 2 2±0 2 (S E) ng/ml (n=5) which were significantly lower than those in controls (p<0 01)

As shown in Fig 2 the mean plasma level of 25 OH D in controls increased significantly from 19 5±3 7 (S E) to 34 0±6 8 (S E) ng/ml after administration of vitamin D (p<0 05) The mean plasma level of 25 OH D in the hepatitis group also increased markedly from 8 0±2 1 (S E) to 22 1±2 6 (S E) ng/ml after vitamin D treatment but it was still lower than that of controls (p<0 05)

The mean plasma levels of 25 OH D in congenital biliary atresia before and after oral administration of vitamin D were 2 2±0 2 (S E) ng/ml and 2 1±0 1 (S E) ng/ml Besides three cases of postoperative congenital biliary atresia showed no change even after parenteral vitamin D was administered as shown in Fig 3 Thus, the vitamin D treatment did not even slightly affect the plasma levels of 25 OH D

DISCUSSION

The fact that osteodystrophy is occasionally noted in patients with liver disease has been reported in some papers (5 10 13 14) In the present study we obtained low plasma levels of 25 OH D in patients with liver diseases

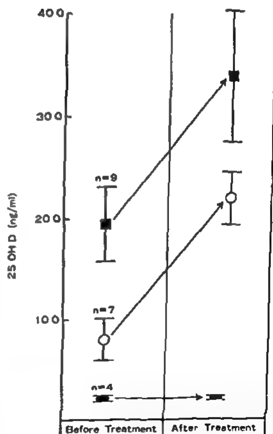


Fig 2 Response of 25 OH D to oral vitamin D treatment for 2 weeks in normal subjects (■) hepatitis group (O) and congenital biliary atresia (●)

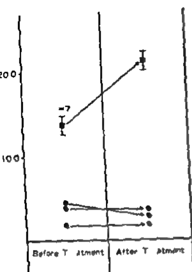


Fig 3 Response of 25-OH D to parenteral vitamin D treatment in normal subjects (■) and congenital biliary atresia (●)

The exogenous vitamin D is absorbed via the lymphatics of the upper gut (3) The absorption of vitamin D via the lymph and bile channels was demonstrated by Avioli et al (2) Also the jejunum is considered to be the primary site of absorption (2 3) After intestinal absorption vitamin D is converted to 25 OH D in the liver However hepatic hydroxylation is easily affected by the activity of microsomal enzyme in hepatic cells In fact the conversion of vitamin D to the metabolite was much slower in cirrhotic liver than in control (4 11) Ponchon et al suggested that rats whose livers have been isolated from circulation do not hydroxylate significant proportions of vitamin D_3 into 25 OH D_3 (9)

The impairment of 25 hydroxylation may be the primary reason why the plasma levels of 25 OH D were much lower in patients with hepatitis than in controls At the same time the failure of intestinal vitamin D absorption caused by the obstruction of bile flow and the difficulty of 25 hydroxylation in progressive liver cirrhosis seems to be the contributing factor to the lower level of 25 OH D in patients with congenital biliary atresia

The increase of plasma levels of 25 OH D after vitamin D treatment suggests that the activity of 25 hydroxylase still remains to some extent in hepatitis In congenital biliary atresia the plasma levels of 25 OH D did not increase even after parenteral vitamin D was administered This finding supports our assumption that the failure of 25 hydroxylation is a major cause of low 25 OH D level in congenital biliary atresia in which severe secondary biliary cirrhosis develops as early as in the third month of life (8)

The results of the present study suggest that patients with neonatal hepatitis should be maintained on supplemental vitamin D intake On the basis of our data a total vitamin D intake of at least 2000 U per day for 2 weeks with subsequent adjustments according to individual requirements seems to be a reasonable initial prophylactic regimen in hepatitis However in the case of congenital biliary atresia administration of 25 hydroxyvitamin D may be preferable

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CARDIOVASCULAR IMPAIRMENT AND PHYSICAL WORKING CAPACITY IN CHILDREN WITH CHRONIC RENAL FAILURE[1]

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ABSTRACT Ulmer H E Greiner H Schuler H W and Scharer K (Departments of Paediatrics and Urology University of Heidelberg Federal Republic of Germany) Cardiovascular impairment and physical working capacity in children with chronic renal failure Acta Paediatr Scand 67 43 1978 —Forty children with chronic renal failure (CRF) on conservative treatment on hemodialysis or after renal transplantation and 22 children representing a non uremic control group were subjected to repeated cardiologic examinations by ECG PCG chest X rays and cycle ergometer exercise tests to monitor signs of uremic heart disease and to evaluate physical working capacity (W_{170}) In the CRF group a progressive impairment of W_{170} was found starting at an early stage of the disease Exercise tolerance was inversely related to the degree of CRF A correlation was also found between W_{170} and renal anemia After starting dialysis W_{170} failed to increase significantly Immediately after dialysis an acute drop in W_{170} occurred Renal anemia was found to be the main pathogenetic factor of uremic heart disease [redacted] children In some cases hypercirculation following arteriovenous fistulae became equally important as a cause of reduced myocardial performance Physical rehabilitation as measured by exercise tolerance tests was better in transplanted than in dialysed children

KEY WORDS Physical working capacity cardiac function chronic renal failure uremic heart disease

Among extrarenal manifestations of chronic renal failure (CRF) cardiovascular complications have been found to be of special importance in children as well as in adults The main causes of death in children treated by regular dialysis or transplantation are of cardiovascular origin (19 24) In adult patients chronic volume load by anemia hypervolaemia and arteriovenous (AV) fistulae as well as chronic pressure load by hypertension and impairment of myocardial metabolism are the main pathological mechanisms of uremic heart disease (UHD) leading to the common clinical and anatomical findings of the heart in CRF (6 7 22 30)

In this study exercise tolerance tests were used in order to obtain objective information on cardiovascular function in children with CRF especially in its early stages The aims of the study were (i) to obtain information on the physical working capacity of children at various stages of CRF and (ii) to determine some of the factors which limit the physical fitness of children with CRF

SUBJECTS AND METHODS

The study was performed on 40 children with CRF defined as a permanent increase of the serum creatinine levels (S_{Cr}) above 177 μmol/l (2 mg/100 ml) The patients were divided into four groups (II to V) according to the

[1] Dedicated to the memory of Professor J Schmier M D of the Department of Experimental Surgery who died on July 29 1976

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Table 2 *Incidence of some results obtained by clinical radiological and ECG examinations given in per cent*

Unfitness ▪ defined as a W_{170} repeatedly below the 25th percentile

Group No of patients	I 2?	II 7	III 13	IV 16	V 4
Tachycardia	14	?8	54	46	50
Dyspnea	9	57	69	3?	0
Unfitness	5	4?	84	6?	25
Cardiomegaly	9	57	78	56	5
ECG changes	0	28	39	50	25
Heart murmurs	32	71	[illegible]	[illegible]	50
Anemia	0	43	100	94	0
Hypertension	5	14	23	0	0
Heart failure	0	14	46	38	0

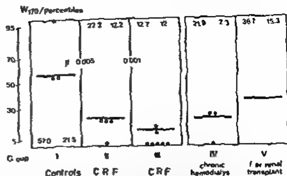


Fig 1 Physical working capacity (W_{170}) in 40 children with chronic renal failure (groups II–V) and 2? control children (group I) The results are presented in percentiles related to the body surface area employing the nomograms of Adams et al (1) Group II S_{CR} 177–443 μmol/l group III S_{CR} above 443 μmol/l

W_{170} of 57 PP In CRF children of groups II and III W_{170} differed significantly with 22 PP and 13 PP respectively (Fig 1) Three of the 7 children in group II—but only 2 of the 13 children in group III—with terminal renal failure had a value above the 25th percentile In dialysed children (group IV) W_{170} was somewhat better but the difference from group III was not significant In contrast almost normal values were found in 4 children examined after renal transplantation

In groups I II and III (but not in the other groups) an inverse correlation was observed between W_{170} and renal function as measured by S_{CR} (Fig 2) A high positive correlation was found between W_{170} and the degree of anemia in groups II and III It is of interest that in 2 children with high S_{CR} in whom W_{170} was relatively high the associated anemia was of a lesser degree than in most other patients

After creation of an AV fistula 4 of 6 children in group III showed a further fall of W_{170} (Table 3) Fig 3 demonstrates the effect of a single hemodialysis on W_{170} in 13 children of group IV The mean value dropped from 22±12 to 11±8 PP immediately after dialysis This fall was observed in spite of a simultaneous reduction of the mean S_{CR} from 867 to 336 μmol/l and the absence of significant changes in the erythrocyte volume

Table 3 *Changes in W_{170} in 6 patients with CRF (group III) immediately before and 6 weeks after creation of an AV fistula expressed in percentile points (PP)*

Patient no	Before fistula operation	After fistula operation
	PP	PP
1	0	0
?	15	5
3	30	30
4	30	3
5	10	5
6	0	5
Mean	?1	11

DISCUSSION

In the paediatric age group maximal tolerance tests which are widely used in adult dialysis patients for the assessment of physical working capacity (2 13 21) are less reliable and not acceptable for ethical reasons On the other hand cycle exercise tests requiring submaximal work loads are usually well tolerated even by sick children (1 2 4 18) Using the Adam s modification (1) of the original method of Wahlund (29) and Bengtsson (4) the results

Table 1 *Clinical findings renal function and hemoglobin in 40 children with CRF (groups II to V) and 22 control children (group I) undergoing physical exercise tests*

S_{CR} Serum creatinine

	Group				
	I	II	III	IV	V
Stage of CRF related to S_{CR} *µmol/l* *mg/100 ml*	<177 <2	177–442 5 2–5	>442 5 >5	Hemodialysis	Transplantation
Number of patients (*n*)	22	7	13	16	4
Age at time of study (years)	12 2±2 6	13 3±0 7	13 5±2 8	14 4±1 9	14 5±2 4
Body surface area (m²)	1 34±0 20	1 19±0 26	1 14±0 25	1 19±0 19	1 4±0 10
S_{CR} micromol/l mg/100 ml micromol/l mg/100 ml	79 7±26 6 0 9±0 3	283 2±79 7 3 2±0 9	654 9±203 5 7 4±2 3	895 0±168 1 10 0±1 9 283 2±106 2[b] 3 2±1 2	106 2±8 9 1 2±0 1
Hemoglobin (g/l)	134±11 3	101±17 0	70±9 0	65±22 2 69±29 0[b]	123±9

Before hemodialysis

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severity of CRF and the mode of treatment (Table 1) They were compared with a control group (I) consisting of 22 children with various renal disorders without or with only minimal impairment of renal function Twenty children with CRF were in the predialysis stage on conservative treatment (CT) 16 children were undergoing regular hemodialysis (HD) three times a week and 4 children were investigated after successful kidney transplantation (TR) In group III 2 children had an AV fistula at the time of the investigation One child was studied on CT and HD and another on HD and then after TR The interval between the first dialysis and the exercise test in group IV ranged between 1 and 14 (mean 6) months

The primary renal diseases of the patients with CRF were nephronophthisis in 10 glomerulonephritis in 7 malformations of the urinary tract in 6 renal hypoplasia in 5 other renal disorders in 3 and unknown in 9 Further information on the study groups is given in Table 1

To evaluate the physical working capacity (*PWC*) the cycle ergometer test was employed as originally described by Wahlund (29) and modified for children by Bengtsson (4) The work load was supplied by a Mijnhard ergometer type FEN (Hellige Freiburg/Breisgau BRD) All children were subjected to three successively increasing work loads for 6 minutes each Stages of work load were related to body weight as indicated by Adams et al (1) In dialysed patients the test was performed immediately before the start of dialysis The heart rate was determined every 2 minutes for 30 seconds by ECG and was plotted in the steady state against the work load performed simultaneously A straight line making the best fit was drawn through the three points The line was extrapolated to a heart rate of 170/min and the corresponding work load was defined as W_{170} (8)

The W_{170} was then plotted against the log of the body surface area and compared with normal children of the same sex and body surface area using the diagrams given by Adams et al (1) The results are expressed in percentile points (PP) which can be read from the diagram with an accuracy of ±5 PP

The cardiothoracic index (CTI) was determined and interpreted by a standard technique (3) Heart rates above 100/min were considered as tachycardia independent of age ECG and PCG were obtained by a 6-channel direct recorder Cardirex 6 (Siemens Erlangen) Blood pressures above the age related normal values according to Lieberman (14*a*) were called hypertensive At the time of the study none of the children received beta blocking agents or other antihypertensives known to influence the heart rate directly Heart failure could only be diagnosed by clinical criteria such as cardiomegaly hepatomegaly tachy dyspnea or pulmonary edema No child was constantly or at the time of the study in heart failure Some children had previously had episodes of heart failure requiring transitory treatment with digitalis

RESULTS

The results of the routine cardiological examinations are presented in Table 2 and the values for W_{170} in Figs 1–3 Children in group I showed a normal distribution with a mean

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Unfitness is defined as a W_{170} repeatedly below the 25th percentile

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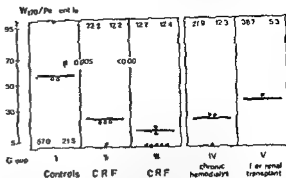


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	15	5
3	30	30
4	30	3
5	10	5
6	0	5
Mean	1	11

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S_{CR}					
micromol/l	79 7±26 6	283 2±79 7	654 9±203 5	895 0±168 1	106 2±8 9
mg/100 ml	0 9±0 3	3 2±0 9	7 4±2 3	10 0±1 9	1 2±0 1
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ECG changes	0	28	39	50	75
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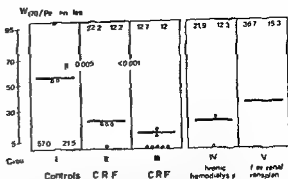


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3	30	30
4	30	3
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agrees well with our observation that for the same degree of glomerular function renal anemia is more severe in children (15)

In adult patients on regular dialysis it was found that the lowered oxygen capacity of patients with renal anemia reduced maximal oxygen uptake during exercise thus stimulating anaerobic metabolism with lactate production (13)

Besides anemia *impairment of myocardial performance* in CRF has been shown to be a further factor responsible for the reduced physical working capacity in adult patients (2 5 18 20 28) We could confirm that this factor also plays a role in children with CRF Previous studies on myocardial function using echocardiography (25) and systolic time intervals (27) in the same series of children presented here indicated that myocardial function was impaired even at rest

Although *lack of physical training* has been proposed as a further factor leading to reduced exercise tolerance in adults with CRF this was not confirmed by repeated exercise tests (13 21) We have previously failed to find any significant difference in W_{170} in a series of children with CRF at the beginning and at the end of a 6 weeks stay in a summer camp in which they undertook intensive physical activity (14)

It was to be expected that the creation of *AV fistulae* especially in larger vessels would increase the work load of the heart (26) In fact children with AV fistulae of the brachial artery lost 10–15% of the cardiac output through the shunt (16) On the other hand AV fistulae in adult patients have only rarely been regarded as a limiting factor for the circulatory function (21) and usually in association with the presence of an external shunt

Our investigations have demonstrated that the W_{170} is not significantly better in children on regular *hemodialysis* than in the predialytic stage of advanced CRF We believe that any improvement due to dialysis is masked by the similar severity of anemia both in the dialysis group (IV) and the non-dialysed group (III) It must be stressed that our studies were restricted to children undergoing haemodialysis for a relatively short period Further studies should analyse the long term effect of dialysis on the cardiovascular system over a period of years especially in relation to the development of anemia Investigations in adults suggest that hemodynamic changes are unaltered by long term dialysis (11)

The fall in W_{170} usually observed immediately after the end of a dialysis session is probably due to the contraction of the extracellular volume associated with a drop in blood pressure and an increase in heart rate (7 16)

In some cases signs of *acute coronary insufficiency* develop during dialysis and cause severe changes in the ECG Myocardial hypoxia might be related to metabolic alkalosis which can occur during dialysis and is known to increase the affinity of hemoglobin to oxygen thereby reducing the oxygen delivery to tissues (23)

In the 4 children examined by us after *transplantation* exercise tolerance was markedly better than in children undergoing regular dialysis It must be stressed that in these transplanted children anemia had resolved completely but AV fistulae were still patent indicating again the importance of a sufficient red cell mass for the maintenance of physical working capacity The better exercise tolerance of transplanted children compared with those on hospital dialysis is likely to be one reason for their higher degree of rehabilitation (19)

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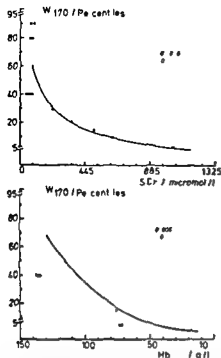


Fig 2 Correlations (*r*) between W_{170} and serum creatinine levels (upper part) and between W_{170} and serum hemoglobin (lower part) in 20 children with CRF in the pre dialytic stage (groups II and III) compared with 22 control children (mean values expressed by the curves)

are expressed in percentile points making it possible to compare the values of boys and girls at various stages of development Just as with other methods variations caused by different degrees of physical inactivity unfortunately cannot be eliminated In a previous study this was proved to be of minor importance (14)

The results of the physical exercise tests performed in this study show that in children with CRF an impairment of physical working capacity (W_{170}) is already present at an early stage when subjective symptoms such as dyspnea can hardly be verified Furthermore a progressive impairment of W_{170} occurring in the course of CRF was also found in this investigation In terminal CRF with serum creatinine levels above 445 μmol/l W_{170} was found to be reduced in 15% more patients than might be suggested by the incidence of dyspnea (Table 2) By institution of regular dialysis mean W_{170} was only slightly improved

The mean value of 22 PP for W_{170} found in children with early CRF (group II) is comparable to results obtained in children with hemodynamically significant aortic or pulmonary stenosis (8)

Our study shows that compared with adult patients with CRF (7), cardiomegaly changes in the ECG, dyspnea and precordial pain are less frequent in uremic children Apparently, during childhood these changes occur later in the course of the disease This difference might be explained by the lower incidence of severe hypertension and vascular disease in children with CRF (17) perhaps thereby causing less myocardial damage than in adults

In this study *anemia* was found to be the main pathogenetic mechanism for uremic heart disease By contrast, adult uremic anemia seems to be less important as a cause of cardiovascular impairment in CRF (6 7 11) although transfusions of packed red cells may improve maximum exercise tolerance (21) Compared with the observations of Del Greco et al (7) in adults with CRF resting tachycardia and cardiac murmurs seem to be less frequent in our pediatric series This difference in the incidence of cardiovascular impairment between adults and children

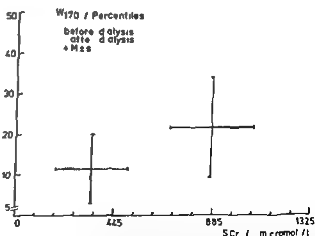


Fig 3 W_{170} before and after a single dialysis session in 13 dialysed children with CRF (group IV) plotted against the corresponding serum creatinine levels (S_{CR}) The crosses represent the mean ±2 S D of W_{170} and S_{CR} before and after dialysis

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LIPOLYTIC ACTIVITY IN MILK FROM MOTHERS OF UNJAUNDICED INFANTS

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ABSTRACT Odievre M and Luzeau R (Unite de Recherche d Hepatologie Infantile I N S E R M U 56 and Clinique de Pediatrie Universite Paris Sud Hopital d Enfants F 94270 Bicetre) Lipolytic activity in milk from mothers of unjaundiced infants Acta Paediatr Scand 67 49 1978—Lipoprotein lipase activity and free fatty acid concentrations are measured in samples of milk collected from 50 mothers of infants without prolonged neonatal jaundice In many samples the values are in the same high range as observed in all samples collected from nine mothers of jaundiced infants These findings are discussed with relevance to the problems of breast milk jaundice and factors controlling the milk secretion

KEY WORDS lipoprotein lipase milk human infant jaundice neonatal

Prolonged neonatal unconjugated hyperbilirubinemia is occasionally associated with breast feeding (2 13) A causal relationship between jaundice and breast feeding has been inferred from the rapid disappearance of jaundice after cessation of breast feeding and the inhibitory effect of milk on the *in vitro* conjugation of bilirubin (2) A 3 alpha 20 beta pregnane-diol has been found in inhibitory samples of milk and has been demonstrated to inhibit the o aminophenol conjugation by guinea pig liver microsomes (2) However doubt has been cast on the role of this steroid in the etiology of jaundice because it was unable to inhibit bilirubin conjugation by human livers (1)

More recently it was found that free fatty acids also have an inhibitory effect on *in vitro* bilirubin conjugation (3 8 9) Fresh milk samples from mothers of jaundiced infants have an increased lipoprotein lipase activity and a normal concentration of free fatty acids progressive release of free fatty acids above 1 mEq/l/day is observed when the milk samples are stored at +4 C for 3–5 days at which time *in vitro* bilirubin conjugation is inhibited by 50% or more (12) Pre heating at +56 C for 15 min of the milk samples before the storage prevents such a release (11) Similar inhibitory effect of free fatty acids on BSP binding to cytoplasmic Z protein has also been documented (6) Additional evidence for a relation between the inhibitory effect of free fatty acids and the prolonged neonatal jaundice is obtained by the rapid disappearance of hyperbilirubinemia after substitution with pre heated milk (11)

However the mechanism by which an increased concentration of free fatty acids in human milk can interfere *in vivo* with bilirubin metabolism remains unclear additional difficulties come from several reports of occasional inhibition with milk from mothers of unjaundiced infants (2 4)

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mEq/l/day of free fatty acids (The amount of free fatty acids released in milk samples of the control group is above 3 0 mEq/l/day)

DISCUSSION

The results obtained in the present study show that a lipoprotein lipase activity progressively appears in the milk from many mothers of un jaundiced infants The relative percentage of milk samples containing a large amount of free fatty acids after storage also increases with the time of collection after birth In both studies the maximum percentage of high values is ob served on the 20th post partum day Similar high values are observed in all samples of milk collected at any time from mothers of infants presenting with breast milk jaundice It has previously been demonstrated that milk samples releasing more than 1 mEq/l/day of free fatty acids after storage inhibit *in vitro* bilirubin conjugation (10) this finding has been confirmed in the present study when a few milk samples collected from mothers of unjaundiced and jaundiced infants were tested after storage using rat liver microsomes as a source of bilirubin glucuronyl transferase

These results do not give any clue as to the mechanism involved in breast milk jaundice They also cast doubt on the validity of sup porting a diagnosis of breast milk jaundice by the mere measurement of inhibitory activity such a diagnosis has to be confirmed by the disappearance of jaundice after weaning or substitution with preheated milk Finally we urge the testing of all presumed normal human milk samples before using them as *in vivo* sub stitutes for pathologic maternal milk In cidentally it must be added that none of the milk samples collected from the mothers of jaundiced infants and tested just after collec tion inhibited the *in vitro* bilirubin conjugation a finding consistent with the assumption that 3 alpha 20-beta pregnane-diol is not the in hibitory substance

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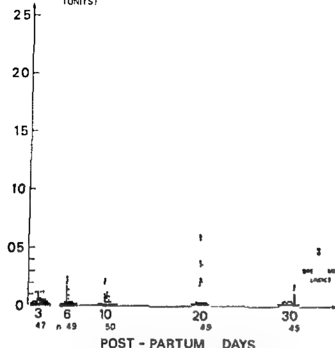


Fig 1 Lipoprotein lipase activity in fresh milk samples collected on different post partum days (the right column corresponds to values measured in the control group)

The present study has been undertaken to *determine lipoprotein lipase activity in milk* from mothers of unjaundiced infants

MATERIAL AND METHODS

Milk was obtained by expression from 50 randomized mothers whose infants had no prolonged neonatal jaundice Milk samples were collected in the middle of day on the 3rd 6th 10th 20th and 30th post partum days in 45 mothers and only on the 3rd 6th 10th and 20th post partum days in 5 others who had insufficient lactation Milk samples collected at any time from 9 mothers of infants with breast milk jaundice were used as controls

Lipoprotein lipase activity was measured in all fresh samples and was *expressed as units/ml (one unit repre* sents the amount of enzyme that releases one μEq of free fatty acid per min at 37°C) each assay mixture con*sisted of 0 25 ml of 1 35 M Tris Hcl* pH 8 1 0 6 ml of 18 7% bovine serum albumin in 0 154 M NaCl pH 8 1 0 4 ml of human serum 0 05 ml of triglyceride emulsion (*Intralipid* 10%) 0 05 ml of heparin (20 I U per ml made up in water) 0 1 ml of milk the final volume was adjusted to 2 5 ml with 0 154 M NaCl

Free fatty acid concentration was measured in each fresh sample and in one aliquot immediately stored at +4 C on the 3rd to 5th day of storage difference be tween the two values was considered as the amount re leased during storage and was expressed as mEq/l/day All the methods used have been previously described (9 11 12)

RESULTS

1 Lipoprotein lipase activity is shown in Fi[g] 1 It is above *0 15 units/ml* in 2, 12 19 31 an[d] 19 samples collected at the 3rd 6th 10th 20[th] and *30th post partum days* respectively [It] remains below this value in samples collecte[d] from the 5 mothers with insufficient lactatio[n] (The enzyme activity is above 0 40 units/ml [in] the control group)

2 The amount of free fatty acids release[d] after storage is shown in Fig 2 It is abov[e] *1 mEq/l/day* in 4 12 26 35 and 25 sample[s] collected at the 3rd 6th 10th 20th and 30t[h] post partum days respectively The sample[s] of milk collected from the 5 mothers with in sufficient lactation all release less than

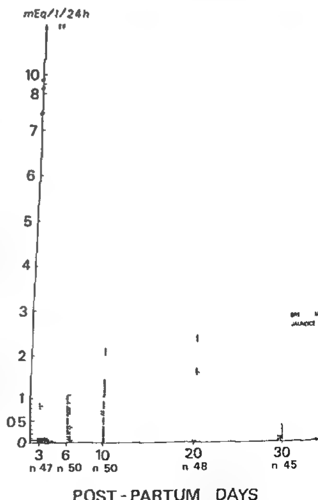


Fig 2 Amount of free fatty acids released during storage at *+4 C of milk samples collected on different post* partum days (the right column corresponds to values measured in the control group)

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PROGNOSIS OF NEONATES WITH SYMPTOMATIC RESPIRATORY INSUFFICIENCY SURVIVING WITH THE AID OF VENTILATOR THERAPY

J KAMPER

From the Department of Neonatology Rigshospitalet University of Copenhagen Denmark

ABSTRACT Kamper J (Dept of Neonatology Rigshospitalet University of Copenhagen Denmark) Prognosis of neonates with symptomatic respiratory insufficiency surviving with the aid of ventilator therapy Acta Paediatr Scand 67 53 1978 —Twenty-one survivors representing all survivors of neonatal symptomatic respiratory distress treated with intermittent positive pressure ventilation have been followed up in an age of 2 9 to 7 0 years The chance of survival proved to be relatively favourable in infants ventilated for perinatal pneumonias and unfavourable in infants ventilated for haemorrhagic diseases and respiratory insufficiency secondary to surgical conditions At the follow-up half of the children presented with neurological symptoms but only 10% were found severely handicapped One infant had a tracheostomy due to a laryngeal stenosis while none developed broncho-pulmonary dysplasia The late prognosis seemed unfavourable when the children had suffered from severe birth asphyxia and in infants ventilated for prolonged recurrent apnoeic spells The relation between the clinical indications for ventilator therapy and later outcome is obscured however by a vast number of complicating perinatal events

KEY WORDS Newborn infants respiratory insufficiency artificial respiration cardiac arrest neurological findings IQ determinations

While the benefits and risks concerning ventilator therapy of newborns with idiopathic respiratory distress syndrome or IRDS (1) are quite well documented there are considerably fewer publications dealing with the short and long term prognosis in newborns ventilated for other reasons Swyer (21) and Strang (20) have both reviewed the theoretical basis for ventilator therapy in a number of symptomatic forms of respiratory distress in newborns and in an extensive survey in 1971 Daily & Smith (5) estimated mean survival rates in this category of newborn infants by major clinical diagnoses The impression given by available long term follow up studies (6 10 17) is that the developmental prognosis in general is the same in infants ventilated for idiopathic and symptomatic respiratory distress syndrome (SRDS) but as only about 30 survivors with SRDS were included final conclusions cannot be drawn

In order to further elucidate the prognosis in these infants I present in the following report survival rates and follow up results of infants treated with intermittent positive airway pressure because of SRDS over a 5 year period

MATERIALS AND METHODS

Early treatment

From 1966 to 1971 a total of 306 infants were ventilated with Bird ventilator Mk II or III Some 199 infants had IRDS (11) and 107 had other respiratory problems (Table 1) Of the latter 27 infants survived ventilator therapy but 6 died at a later time from causes shown in Table 2

Only few of the survivors had been without pregnancy or birth complications (Table 3) Of the 11 boys and 10 girls 13 were born in Rigshospitalet and 8 were referred from other hospitals as emergency cases The gestational ages ranged between 29 and 40 weeks and the birth

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Table 1 *Clinical indications for artificial ventilation*

	Dead		Survived	
	On vent	After vent	*n*	%
Pneumonia perinatalis	3	1	6	60
Apnoea primaria	7	1	3	23
Apnoea paroxysmatica	10	1	4	27
Hydrops foetalis (Rhesus imm)	15	0	3	17
Hydrops foetalis (idiopathic)	2	(1)	(1)	(25)
Sepsis–meningitis	7	1	2	20
Diathesis haemorrhagica	4	0	0	0
Mb metabolicus congenitus	2	0	1	33
Maleformatio (postoperative)	18	1	2	10
Maleformatio (non operative)	14	1	0	0
Total	80	6	21	20

Included in apnoea primaria

weights between 1 080 and 3 900 g Five infants are listed as cases of perinatal pneumonia which includes meconium aspiration as well as infectious pneumonias as to distinguish between these conditions is difficult and uncertain in survivors (1) After delivery 14 infants were resuscitated and 6 still needed artificial ventilation on arrival in the neonatal department Of these infants 3 are listed as primary asphyctic 2 as perinatal pneumonias (nos 82 & 123) and 1 as hydrops foetalis due to rhesus immunization (no 24) Infants with hydrops foetalis were also treated with peritoneal dialysis as earlier described by Nathan (16)

One infant transferred from another hospital was apnoeic and depressed on arrival and ventilator treatment had to be started immediately The principal diagnosis proved to be hypoglycaemia but in spite of the administration of intravenous glucose the ventilator therapy had to be continued for 35 hours A total of 6 infants had cardiac arrests but were successfully resuscitated 3 before and 3 after ventilator therapy had been instituted

Indications technique and mode of ventilation employed in this department have been described in earlier (3 4 9 14) and recent publications (11 12) Most infants in this heterogeneous series were ventilated because of apnoea or insufficient respiration with general cyanosis in spite of pure oxygen administration However acid–base status was measured with the Astrup technique (19) prior to mechanical ventilation in 15 infants The most abnormal mean values for P_{CO_2} and pH were 11 86 kPa (range [illegible] 66–19 99) and 7 07 (6 77–7 30) which illustrates the grave prognosis of these infants without assisted ventilation Details concerning ventilator and oxygen therapy are given in Table 4

Follow up procedure

All survivors could be included in the follow up examination The parents returned a questionnaire containing anamnestic information and the children were then evaluated in a specially established follow up clinic by the author One child was examined by specialists at another institution and included per report

Neurological and ophthalmological examination growth measurements auditory screening developmental and psychological testing were carried out along the same lines as in the group of IRDS survivors (11) In brief children below 5 years of age had their physical examination supplemented with the Denver developmental screening test (DDST) and Bo Ege s linguistic test and those over 5 were evaluated by a psychologist with Wechsler s intelligence tests for preschool and school children (WPPSI and WISC)

Measurements of capillary oxygen saturation and acid base status were obtained in 20 children sitting at rest and breathing atmospheric air The chest X rays which were performed on all children were evaluated blind together with 129 of those belonging to the IRDS survivors and their controls (12)

RESULTS

The most important follow up findings are listed in Table 3 In the following the findings will be reported under the headings of growth psycho motor development and intellectual status neurological findings and pulmonary morbidity and status

Growth measurements

Compared with recent Scandinavian standards (8) the average weight was found to be 1 4 kg below mean and the average length 1 3 cm below mean The average head circumference was 0 7 cm larger however A 5 1 year old girl with tetraplegia was found to be 2 standard deviations below both mean weight and length and a 4 4 year old boy born 10 weeks before term was more than 2 standard deviations below mean A 3 8 year old boy had a head circumference=56 3 which is more than 3 standard deviations above the mean This boy also had a slight monoplegia and may have suffered from a spontaneously arrested hydrocephalus

Psycho motor development and intellectual status

Two children with tetraplegia were unable to walk The remainder walked at an average age of 15 8 months In 11 cases it was possible to

Table 2 *Causes of death by clinical indication for ventilator treatment*

Patient no	Clinical indication	Age at death	Cause of death
107	Hydrops foetalis	7 days	Birth traumatic fracture of cervical spine
16	Perinatal pneumonia	8 days	Progressive respiratory failure
57	*Malformatio cordis congenita*	*9 days*	*Anomalous pulmonary venous return*
238	Meningitis	3 weeks	Meningitis
157	Apnoea paroxysmatica	4 weeks	Bronchopulmonary dysplasia
51	Atresia duodeni operata	5 weeks	Peritonitis

show that the ventilated survivors walked on an average 2 6 *months later than elder siblings* The difference is significant ($p<0\ 01$ Wilcoxon test for independent variables)

13 children tested *with linguistic psycho* motor or formal intelligence tests were normal The 2 children with tetraplegia were clearly mentally retarded Two children presented with dysphasia *and a 4 1 year old boy* was found borderline abnormal with regard to both linguistic and psycho motor performance which could not be explained by specific sensoral or motor handicaps A 4 4 year old girl with hearing loss was also speech retarded and a 3 8 year old boy with monoplegia did not pass the DDST because of abnormal gross motor' and personal–social performance *The mean intelligence* quotient of 9 children aged 5 years or more who were tested with WPPSI or WISC was 113 (range 99–131)

Neurological findings

Slight to severe symptoms of *cerebral palsy* were found in 6 children 2 of whom were severely handicapped (nos *123 & 164)* One of these developed innumerable apnoeic spells necessitating frequent manual and from the 11th–27th day of life mechanical ventilation On a single occasion a pH value=6 77 was measured during an apnoeic spell prior to the ventilator treatment

A 5 6 year *old girl* (no *82) revealed a limited* paraplegia with unaffected intelligence (IQ= 131) She also suffered *from laryngeal stenosis* needing tracheostomy possibly due to an *emergency intubation in the delivery room* Three additional children presented with slight symptoms of cerebral palsy and one was not *able to pass the motor subtests of the DDST*

A 6 2 year old girl operated on a few hours after birth for eventration of the intestines and ventilated postoperatively for only 39 hours *presented with* behavioural symptoms compatible with *minimal brain dysfunction* While the ventilator therapy was uneventful it is remarkable that during the first 8 days she was nourished mainly parenterally with 5 and 10% glucose solutions and that *her intake of* mother s milk was 12–13 *times lower* than the *mean intake* of the whole series during the same period (382 ml/kg birth weight/week) Cicatricial *retrolental fibroplasia* was found bilaterally in one child who was also tetraplegic and monolaterally in another These children were born 10 and 11 *weeks before* term Strabismus was also found in 3 children *One girl born* 10 weeks before term developed bilateral *neurosensoral deafness* needing hearing aid This may be due to neonatal *hyper* bilirubinaemia She also received kanamycin but in a lower dose per kilogram birth weight and for a *shorter time than the mean of* 7 other children who were treated with the same antibiotic

Pulmonary morbidity and status

The previously mentioned girl who had a tracheostomy and a boy with tetraplegia still suffered from recurrent pneumonias *due to in*sufficient coughing mechanisms Two additio-

Table 1 *Clinical indications for artificial ventilation*

	Dead		Survived	
	On vent	After vent	*n*	%
Pneumonia perinatalis	3	1	6	60
Apnoea primaria	7	1	3	23
Apnoea paroxysmatica	10	1	4	27
Hydrops foetalis (Rhesus imm)	15	0	3	17
Hydrops foetalis (idiopathic)	2	(1)	(1)	(25)
Sepsis–meningitis	7	1	2	20
Diathesis haemorrhagica	4	0	0	0
Mb metabolicus congenitus	2	0	1	33
Maleformatio (postoperative)	18	1	2	10
Maleformatio (non operative)	14	1	0	0
Total	80	6	21	20

Included in apnoea primaria

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Table 3 *Obstetrical neonatological and follow up characteristics*

Pat no	Complications in pregnancy or at delivery	Gestational age (wk)	Birth weight (g)	Clinical indication for ventilation	Complicating neonatal events
24	2 3 10 13	35	3 400	Hydrops foetalis	Arrestio cordis convulsions se bili =14 1 (2)[a]
63		36	2 200	Eventratio intestini	
80	14	39	2 250	Hypoglycaemia	Small for dates convulsions
81	1 8 9 10 13	39	1 600	Atresia duodeni	Small for dates
82	1 10 13 14	38	2 700	Pneumonia perinatalis	Arrestio cordis convulsions
108	9 14	40?	2 300	Pneumonia perinatalis	Convulsions
109	9 14	40?	3 300	Pneumonia perinatalis	Convulsions
114	14	40?	3 900	Pneumonia perinatalis	Pneumothorax
118	3 6 7 10 13	31	2 440	Hydrops foetalis	Convulsions se bili =19 5 (3)
123	12 13 14	36	1 950	Pneumonia perinatalis	Small for dates convulsions
125	3 10 13	33	2 200	Hydrops foetalis	Arrestio cordis se bili =20 3 (4)
154	2 11 13	30	1 200	Apnoea paroxysmatica	Se bili =20 1 (2)
156	2	31	2 060	Pneumonia perinatalis	Se bili =17 7 (0)
158	3 13	30	1 500	Apnoea primaria	Convulsions se bili =17 9 (4)
164	2 13	29	1 600	Apnoea paroxysmatica	Arrestio cordis convulsions
181	3 11 13 14	37	2 000	Sepsis (coli)	Se bili =21 3 (4)
191	7 10 12 13	40	2 170	Sepsis (coli)	Arrestio cordis small for dates
206		30	1 550	Apnoea paroxysmatica	
233	4 13 14	28	1 080	Apnoea paroxysmatica	Se bili =18 2 (4)
275	5 10 13	38	3 100	Apnoea primaria	Pneumothorax convulsions
290	1 2 8 12 13	34	3 760	Apnoea primaria	Hydrops arrestio cordis se bili =25 5 (2)
Mean		35	2 298		

1 pre eclampsia 2 bleeding episodes 3 rhesus immunization 4 A–O immunization 5 maternal diabetes mellitus 6 maternal colitis ulcerosa 7 placental insufficiency 8 hydramnios 9 twins 10 Caesarean sectio 11 abnormal vaginal delivery 12 intra uterine asphyxia 13 resuscitation at birth 14 transferral

[a] Maximal serum bilirubin value (se bili) in mg/100 ml Number of exchange transfusions indicated in parentheses

nal children both had pneumonias causing hospitalisation before their third year of life and one also suffered from bronchial asthma The total frequency of children with pneumonias causing hospitalisation (19%) is 7% lower than among survivors ventilated for IRDS in the same period (12) but 12% higher than their controls

Six children presented with chest X ray abnormalities all having grade I line shadows according to the terminology of the Norman score system (15) Two also had mottled shadows and one a large shadow The overall frequency of abnormal chest X rays was 29% or 8% lower than among the above mentioned IRDS survivors *but 13% higher than* their non ventilated controls Chest and heart configuration and size was normal in all The average Norman score was 0 43 against 0 58 among the IRDS survivors Statistical analysis of therapeutical characteristics (Table 4) showed no significant differences between children with and without X ray changes (Wilcoxon test for independent variables) but as in the IRDS survivors a tendency was seen

Table 4 *Therapeutical characteristics*

	Mean	S D	Range
Age at start of ventilation (h)	46	75	0 2–284
Duration of ventilation (h)	105	113	3–456
Duration of oxygen conc >21% (h)	406	334	85–1 292
Duration of oxygen conc ≥70% (h)	160	127	42–539

...ow up investigation	
	Abnormal findings
	Minimal brain dysfunction
	Hypoglycaemic attacks
	Paraplegia stenosis laryngis
	Tetraplegia oligophrenia
	Hypacusis bilateralis
	Monoplegia
	Tetraplegia oligophrenia epilepsy retrolental fibroplasia
	Retarded
i	
i	Monoplegia retrolental fibroplasia in one eye
5	
1	Dysphasia paraplegia
9	Dysphasia
7	

that the group with X ray changes had received both ventilator and oxygen treatment for the longest periods Mean values of capillary oxygen saturation (0 97±0 01) P_{CO_2} (4 39±0 26 kPa) pH (7 44±0 02) and standard bicarbonate (23 7±1 5 mmol/l) did not statistically differ from values obtained in the group of IRDS survivors and their controls when compared by Student s *t* test

DISCUSSION

As indicated in Table 1 survival rates of ventilated SRDS differ greatly for the various types Like others (5 6) we find that the highest survival rates are among infants ventilated for pneumonia and recurrent apnoeic spells The rate in infants with hydrops due to rhesus immunization remained low in spite of preliminary good results with the additional use of peritoneal dialysis and exchange transfusions The extremely low survival rates among infants receiving postoperative support (mainly after corrections for diaphragmatic hernias oesophageal atresias and intestinal malformations) is thought to have been caused by the advanced degree of respiratory failure in the infants selected for ventilator therapy Also the insufficient parenteral alimentation available at that time may have played a significant role

At the follow up evaluation various abnormalities were found among 11 out of 21 survivors only 2 of whom however are thought to be severely and irreversibly handicapped The relationship between the neonatal ventilator therapy and later sequelae is as indicated in Table 3 and illustrated by the case reports very often obscured by a number of complicating perinatal events some of which undoubtedly have influenced the late prognosis

A few conspicuous relationships between neonatal conditions and outcome call for comment however A total of 14 infants were asphyctic at birth and had to be resuscitated Only 2 out of 8 infants who established rhythmic spontaneous respiration within 10 minutes following resuscitation developed symptoms of brain damage as against 5 out of 6 who needed intubation and ventilation for more than 10 minutes which confirms the already demonstrated close correlation between the degree of *birth asphyxia* and later outcome in human infants (7)

One of the two severely handicapped children was ventilated for *recurrent apnoeic spells* Another child from this group also developed sequelae in the form of a slight paraplegia while two survived intact This high incidence of neurological sequelae corresponds to Bacola and co workers (2) findings in a series of small prematures surviving prolonged apnoeic spells with conservative therapy and may be due to the fact that our

infants were not ventilated until after a number of spells one of which as mentioned could be shown to have resulted in severe asphyxia Tsiantos et al (22) have shown that apnoeic spells are only infrequently indicative of an existing or threatening intracranial haemorrhage and therefore we still consider apnoeic spells to be an indication for IPPV in case the spells have not responded to usual methods i e CPAP (13) or *theophyllamine* (18)

Six infants had *cardiac arrest* successfully treated with closed heart massage The late prognosis of infants surviving cardiac arrests in the neonatal period is not known It is therefore interesting to note that the infant (no 125) with the most protracted cardiac arrest lasting 15 minutes was found normal at the follow up This indicates that even a protracted cardiac arrest in neonates may be overcome without resulting brain damage

No survivors developed broncho pulmonary dysplasia except one who died shortly after ventilator therapy Although 29% revealed *chest X ray abnormalities* these were all discrete and presumably without significance as no children had clinical or biochemical evidence of impaired lung function as indicated by oxygen saturation and acid–base status Of course only a further follow up including more sophisticated lung function studies could confirm this

This series illustrates that a significant number of neonates suffer from severe respiratory insufficiency from causes other than surfactant deficiency The prognosis depends greatly on the nature of the underlying disease(s) and the preventilatory condition of the infants i e the more hypoxia acidosis hypoglycaemia hypothermia and hyperbilirubinaemia the worse the outlook Improvements will therefore depend on advances in obstetrics, transport facilities neonatal surgery and parenteral nutrition as well as ventilatory technique, thus allowing a more active approach with earlier intervention in infants with SRDS

Conclusions as to the long term prognosis by diagnosis are not easily drawn due to the small size and the complexity of the material although the figures tend to delineate the asphyctic infants who do not establish spontaneous respiration within 10 minutes of birth and infants with recurrent apnoeic spells as very high risk groups When surveying the whole series however we feel optimistic that only 10% of these heavily burdened and diseased neonates developed permanent severely handicapping cerebral palsy

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LONG TERM PROGNOSIS OF INFANTS WITH SEVERE IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

I *Neurological and mental outcome*

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ABSTRACT Kamper J (Department of Neonatology Rigshospitalet University of Copenhagen Denmark) Long term prognosis of survivors of severe idiopathic respiratory distress syndrome I Neurological and mental outcome Acta Paediatr Scand 67 61 1978 —76 out of 77 children surviving IRDS with the aid of intermittent positive pressure ventilation have been followed up by the age 2 6–7 6 years together with 68 matched controls Moderate or severe neurological developmental or mental abnormalities were present in 17 % of all IRDS survivors Statistical comparison of the matched pairs of IRDS survivors and controls revealed no significant differences in the prevalence of abnormalities In the IRDS survivors the occurence of cerebral palsy related to prematurity while the development of psycho-motor and mental retardation related to low birth weight and low milk intake during the first week suggesting that both prenatal and postnatal growth retardation may have been of importance Statistical analysis of a number of preventilatory and ventilatory parameters did not show significant differences between these groups of IRDS survivors and the remainder Ventilator treatment is recommended as a promising adjunct to the therapy of severe IRDS in centers where the necessary experience and equipment is at hand

KEY WORDS Newborn infants respiratory distress syndrome artificial respiration neurological findings IQ determinations

Since Donald & Lord (8) in 1953 introduced the ventilator in the treatment of newborn infants with idiopathic respiratory distress syndrome (IRDS) this mode of treatment has become widely adopted While technical procedures survival rates and early complications have been dealt with in several reports (2 3 21 27 34 36 37) the long term prognosis seems less well elucidated The few available reports indicate that a proportion of ventilated IRDS survivors may develop neurological handicaps especially when positive pressure ventilators have been employed (7 20 22 33) But it remains to be settled whether the prevalence of neurological and mental handicaps is higher than expected from the low mean gestational age of these children

The following report deals with the development neurological and mental status of 76 ventilated IRDS survivors between 2 6 and 7 6 years of age and for comparison a group of children matched with regard to a number of factors known to influence the early morbidity and development (9 10 23)

MATERIALS AND METHODS

IRDS survivors

At the Neonatal Department in Rigshospitalet intermittent positive pressure ventilation (IPPV) was introduced in the treatment of IRDS in April 1966 and till August 1971 a

Table 3 *Results of the Denver Developmental Screening Test in 42 ventilated IRDS survivors and matched controls*

Number and per cent of infants with abnormal performance

	Ventilated		Controls	
	No	%	No	%
Personal-Social	10	24	8	19
Fine motor adaptive	7	17	4	10
Language	8	19	1	2
Gross motor	11	26	8	19
Overall	6	14	3	7

$\chi^2=4.479$ d f $=1$ $p=0.034$

within the last 3 months Further details referring to this part of the examination are given with the results The physical and neurological investigation was supplemented by measurements of height weight and head circumference Cooperative children had their hearing and vision screened In case of documented or suspected abnormalities the children were evaluated further by audiologists and ophthalmologists All children who came to follow up [illegible] Rigshospitalet were also evaluated with regard to cardio-pulmonary status The results of this part of the investigation are published separately (25)

Children aged below 5 years at the beginning of the follow up were examined with the revised Denver Developmental Screening Test (DDST) which allows a distinction between "Personal-social" "Fine motor adaptive" "Language" and "Gross motor" performance (15 16) Vocabulary and linguistic qualities were evaluated further with the Danish Bo Ege linguistic test no I which is standardized for children between 3 and 7 years (11) Children aged 5 years or more were evaluated blindly by a psychologist with Wechsler s intelligence tests for pre school and school children (WPPSI and WISC) which give a broad impression of the children s verbal and performatory abilities (4) Moreover these children were tested with Goodenough Harris Drawing Test and Bender Gestalt Test Gross motor abilities of this age group were examined by trying out the ability [illegible] catch a tennis ball 3 out of 3 times at various distances and to balance standing on one leg and hopping forward

Statistics

In comparisons of IRDS survivors and controls only matching children have usually been used The actual number compared is always indicated Differences of groups have been evaluated employing the chi square test with the Yate s correction factor and differences of means employing Student s t test

RESULTS

Growth

Comparisons of mean values for height weight and head circumference showed no significant differences between IRDS survivors and controls Compared to values predicted from recent Swedish growth curves (12) the height and weight of the IRDS survivors on an average were retarded 1 6 cm and 0 8 kg and the head circumference accelerated 1 0 cm The deviations were all within one standard deviation of the predicted mean values

Psycho motor development

Early *motor development* was estimated from the parental dating of the first step without support among 62 match pairs Three IRDS survivors with cerebral palsy were not able to walk alone Of the remainder 42 did not walk without support until after 13 months old All controls walked without support and only 31 children did not walk until after the 13th month The difference is significant ($p<0.02$)

Table 4 *Intellectual performance in ventilated IRDS survivors and matched controls of five years of age and older*

	Ventilated	Controls	[illegible]
WISC/WPPSI	No 24	No 24	
Verbal score ($\bar{x}\pm1$ S D)	98 7±15 4	105 6±15 0	N s
Performance score ($\bar{x}\pm1$ S D)	105 6±19 0	11? 2±13 4	N s
Total score ($\bar{x}\pm1$ S D)	10[illegible] 1±16 9	109 7±13 4	N s
Bender Gestalt Test	No 6	No 6	
Subnormal	7	4	N s
Goodenough Harris Test	No 26	No 6	
Subnormal	6	3	N s

Table 1 *Causes of death*

	Age at death
Intracerebral haemorrhage	5 days
Pneumonia	7 days
Septicemia	2 weeks
Bronchopulmonal dysplasia (4)	4 5 6 7 weeks
Encephalomalacia (porencephalia)	13 weeks
Cot death	9 months

total of 199 infants were treated this way 113 infants died on the ventilator and 9 later (Table 1)

*Diagnostic criteria for IRDS methods of and indica*tions for ventilator therapy and early results from this department have been published in previous reports (2 3 17 26) In brief IRDS was diagnosed when an infant developed progressive respiratory failure and the chest X ray showed the characteristic changes (30) The infants were treated by Bird ventilators Mk 8 or 10 following naso-tracheal intubation with Portex tubes no 2½–3½ While technique and mode of ventilation have remained fairly unchanged through the period the indications for ventilation were changed In the beginning most infants were *ventilated when Pa_{O_2} after a hyperoxia test (1) had* fallen below 6 66 kPa (50 mm) In total 15 IRDS survivors were ventilated taking this indication their average Pa_{O_2} being 5 73 kPa (43 mm) During the last four years of the period we usually employed a combined clinical bio chemical *indication (51 IRDS survivors) demanding gen* eral cyanosis and symptoms of exhaustion plus a respira tory acidosis with pH values below 7 20 and P_{CO_2} values above 9 33 kPa (70 mm) *The most abnormal mean values* in this group of infants before ventilation were pH=7 17 and P_{CO_2}=10 66 kPa *(80 mm) Ten IRDS* survivors ad mitted critically ill were ventilated without prior meas urements of blood gases The average IRDS survivor in *this material received IPPV for 6 days (2nd to 8th) and* increased inspiratory oxygen for nearly 18 days Concen trations from 70 to 100% were given for 9 days (25)

The infants received early infusions of 10% glucose or invertose solutions through an umbilical artery or vein catheter From the 2nd or 3rd day of life the infants were given gavage feedings with human milk The average IRDS survivor was given 305 ml of milk per kg birth weight (n=73) in the first week of life Feeding through a gastrostomi was never attempted Infants with indwelling *umbilical catheters were given ampicillin (100 mg/kg/day* i m) or kanamycin (20 mg/kg/day i m) Following naso-tracheal intubation the infants were treated prophylacti cally with a combination of ampicillin and polymyxin B (2 mg/kg/day i m) Before discharge the infants had a routine ophthalmoscopic investigation which was re peated in case of suspect findings

One surviving infant had been adopted and was there *fore excluded leaving a total of 76 IRDS survivors to* participate

Matched controls

A group of 70 children with no history of IPPV could be *selected as paired controls matching the IRDS survivors* in the following respects

1 Born at *Rigshospitalet or admitted* to the Neonatal Department
2 *Sex*
3 *Day of birth ± 3* months
4 Gestational age ±1 week (when calculable from men strual anamnesis)
5 Birth weight ± 300 grams (when gestational age was not calculable)
6 *First born/later born*
7 Single born/double born
8 Socio-economic status (28)

Two of these children were lost to follow up Hence the report deals with [illegible] pairs of matched IRDS survivors *and controls as well as 8 unmatched IRDS survivors* Statistical comparison of IRDS survivors and controls shows only small differences in mean birth weight and mean gestational age (Table 2)

Follow up procedure

143 children were examined by the author 131 at the fol low up clinic and 12 at other clinics or at home One *child living abroad participated only with a question* naire

A questionnaire was forwarded to the parents to allow *them at home to fill in anamnestic details On the day of* the follow up the information was scrutinized and sup plemented with a standardized interview concerning a number of psychosomatic and behavioural symptoms

Table 2 *Neonatal characteristics and age at follow up in 68 ventilated IRDS survivors and matched controls*

Gestational age only obtainable in 6? *matched pairs* [illegible] s *=not significant*

	Ventilated		Controls		
	$\bar{x}$	(Range)	$\bar{x}$	(Range)	p
Birth weight (g)	2 473	(1 250–4 510)	2 466	(1 200–4 000)	N s
Gestational age (weeks)	34 7	(29–40)	34 9	(30–40)	N s
Age at follow up (years)	4 8	(2 6–7 6)	4 8	(2 6–8 0)	N s

Table 3 *Results of the Denver Developmental Screening Test in 42 ventilated IRDS survivors and matched controls*

Number and per cent of infants with abnormal performance

	Ventilated		Controls	
	No	%	No	%
Personal–Social	10	24	8	19
Fine motor adaptive	7	17	4	10
Language	8	19	1	2
Gross motor	11	26	8	19
Overall	6	14	3	7

$\chi^2=4\ 479$ d f = 1 $p=0\ 034$

within the last 3 months Further details referring to this part of the examination are given with the results The physical and neurological investigation was supplemented by measurements of height weight and head circumference Cooperative children had their hearing and vision screened In case of documented or suspected abnormalities the children were evaluated further by audiologists and ophthalmologists All children who came to follow up in Rigshospitalet were also evaluated with regard to cardio-pulmonary status The results of this part of the investigation are published separately (15)

Children aged below 5 years at the beginning of the follow up were examined with the revised Denver Developmental Screening Test (DDST) which allows a distinction between Personal–social Fine motor adaptive Language and Gross motor performance (15 16) Vocabulary and linguistic qualities were evaluated further with the Danish Bo Ege linguistic test no 1 which is standardized for children between 3 and 7 years (11) Children aged 5 years or more were evaluated blindly by a psychologist with Wechsler s intelligence tests for pre school and school children (WPPSI and WISC) which give a broad impression of the children s verbal and performatory abilities (4) Moreover these children were tested with Goodenough Harris Drawing Test and Bender Gestalt Test Gross motor abilities of this age group were examined by trying out the ability to catch a tennis ball 3 out of 3 times at various distances and to balance standing on one leg and hopping forward

Statistics

In comparisons of IRDS survivors and controls only matching children have usually been used The actual number compared is always indicated Differences of groups have been evaluated employing the chi square test with the Yate s correction factor and differences of means employing Student s *t* test

RESULTS

Growth

Comparisons of mean values for height weight and head circumference showed no significant differences between IRDS survivors and controls Compared to values predicted from recent Swedish growth curves (12) the height and weight of the IRDS survivors on an average were retarded 1 6 cm and 0 8 kg and the head circumference accelerated 1 0 cm The deviations were all within one standard deviation of the predicted mean values

Psycho motor development

Early *motor development* was estimated from the parental dating of the first step without support among 62 match pairs Three IRDS survivors with cerebral palsy were not able to walk alone Of the remainder 42 did not walk without support until after 13 months old All controls walked without support and only 31 children did not walk until after the 13th month The difference is significant ($p<0\ 02$)

Table 4 *Intellectual performance in ventilated IRDS survivors and matched controls of five years of age and older*

	Ventilated	Controls	p
WISC/WPPSI	No 24	No 24	
Verbal score ($x\pm1$ S D)	98 7±15 4	105 6±15 0	N s
Performance score ($x\pm1$ S D)	105 6±19 0	112 2±13 4	N s
Total score ($x\pm1$ S D)	10? 1±16 9	109 7±13 4	N s
Bender Gestalt Test	No 6	No 26	
Subnormal	7	4	N s
Goodenough Harris Test	No 26	No 26	
Subnormal	6	3	N s

Table 5 *Birth weight gestational age most abnormal P_{CO_2} and pH before ventilation and intake of milk in the first week of life in IRDS survivors with and without cerebral palsy (CP) respectively mental/psychomotor retardation (MR)*

One infant with both CP and MR was ventilated without prior measurements of blood gases

	+CP			−CP			+MR			−MR		
	N	*x*	*1 S D*	*N*	*x*	*1 S D*	*N*	*x*	*1 S D*	*N*	*x*	*1 S D*
Birth weight (g)	5	1 778	387	71	2 481	640	12	2 077[a]	440	64	2 501	663
Gestational age (weeks)	4	31 4	1 9	65	34 9	2 7	9	34 1	3 7	60	34 8	[illegible]
P_{CO_2} (kPa)	4	12 79	4 79	62	10 13	1 99	11	11 59	3 33	55	10 13	1 99
pH	4	7 12	0 12	62	7 18	0 06	11	7 17	0 14	55	7 18	0 07
Milk intake (ml/kg birth weight)	5	195	98	68	314	155	12	216[b]	117	61	323	155

$p<0\ 02$ [b] $p<0\ 05$

Gross motor tests for children above 5 years of age showed no significant differences

Retrospective dating of *speech development* is more inaccurate We therefore made a relative estimation comparing children in each of the groups to their next elder sibling The *parents of 35 IRDS survivors with elder siblings* stated that the speech development had been *considerably* slower in 19 cases (56%) This was only the case among 9 out of 31 controls (29%) but the difference may be due to chance ($p>0\ 05$) *42 match pairs between 2 6* and 5 4 years of age were examined with the *DDST* (Table 3) Seven IRDS survivors (one unmatched) and 3 controls had an abnormal overall performance The difference is not significant The IRDS survivors performed the linguistic subtest significantly poorer than the controls The average scores in *Bo Eges linguistic test* in 37 testable IRDS survivors and their matches were 27 16 (range 4–39) and 29 24 (range 12–41) This difference is not significant, however

Results of psychometric testing

Bender Gestalt Test and Goodenough Harris Drawing Test were carried out in 26 match pairs and WISC or WPPSI in 24 of at least 5 years of age (Table 4) Although the controls generally performed a little better than the IRDS survivors no differences reached statistical significance The analysis of the subtests common to WISC and WPPSI (information comprehension arithmetic : similarities, vo

Fig 1 Percentage of psycho-somatic symptoms in 65 matched pairs of IRDS survivors and controls None of the differences is significant

Fig 2 Percentage of behavioural characteristics in 65 matched pairs of IRDS survivors and controls None of the differences is significant

Table 6 *Neurological developmental and mental abnormalities in 68 ventilated IRDS survivors and matched controls*

	Ventilated		Controls	
	No of abnormalities	No of children	No of abnormalities	No of children
Total number without abnormalities		47		57
Cerebral palsy	5		3	
Epilepsy	3		0	
Hydrocephalus	3		0	
Retrolental fibroplasia	3		1	
Bilateral hearing loss	7		2	
Speech defect	3		2	
Abnormal DDST	6		3	
IQ<85 points	5		1	
Emotional disturbance	5		3	
Total number with abnormalities				
Minor		10		6
Moderate–severe		11		5

$\chi^2=4.71$ d.f. = $p=0.17$

cabulary picture completion and coding) revealed no significant differences either Five IRDS survivors obtained IQ s lower than 85 points against one child in the control group

Twelve IRDS survivors with either low IQ or abnormal performance with the DDST were grouped together and analysed with regard to gestational age birth weight most abnormal P_{CO_2} and pH values recorded prior to ventilation and milk intake during the first week of life As indicated in Table 5 this group was 424 g lighter at birth ($p<0.05$) but only 0.7 weeks more premature than the other IRDS survivors ($p>0.05$) Nine out of 12 infants with known gestational ages had a mean birth weight equal to 2044 grams corresponding to the 20th centile for gestational age (5) The 12 infants with mental or psycho motor retardation had a significantly lower mean intake of milk during the first week (*Table 5*) while differences in P_{CO_2} and pH values did not reach statistical significance Nor did the duration of ventilator treatment the frequency of pneumothoraces or tube complications

Emotional status

Interviews concerning psycho-somatic and behavioural symptoms which may reflect emotional disorders were completed in 65 matched pairs The prevalence of symptoms is shown in Figs 1 and 2

Only a minority in both groups was completely free of symptoms The statistical analysis revealed no significant differences neither in prevalence of any single symptom nor in the total number of children affected in the two groups Five children in both groups needed professional psychological or psychiatric assistance

Neurological status

Five IRDS survivors suffered from *cerebral palsy* Two of these had severe tetraplegias with oligophrenia and epilepsy, 2 had diplegias and moderate mental retardations (IQ 70 and 84) and the last child a slight paraplegia without intellectual deficiency Table 5 shows that these 5 children at birth were relatively small and premature but with appropriate weight for gestational age One of these infants was admitted critically ill due to tube occlusion Another child with tetraplegia in addition to the ventilator treatment went through a complicating ileal perforation resulting in two laparotomies As indicated in Table 5 the

Table 5 *Birth weight gestational age most abnormal P_{CO_2} and pH before ventilation and intake of milk in the first week of life in IRDS survivors with and without cerebral palsy (CP) respectively mental/psychomotor retardation (MR)*

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	N	*x*	1 S D	*N*	*x*	1 S D	*N*	*x*	1 S D	*N*	*x*	1 S D
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Gestational age (weeks)	4	31 4[a]	1 9	65	34 9	2 7	9	34 1	3 7	60	34 8	2 7
P_{CO_2} (kPa)	4	12 79	4 79	62	10 13	1 99	11	11 59	3 33	55	10 13	1 99
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[a] $p<0.02$ [b] $p<0.05$

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$\chi^2=4$ [illegible]1 d f =[illegible] $p=0$ 12

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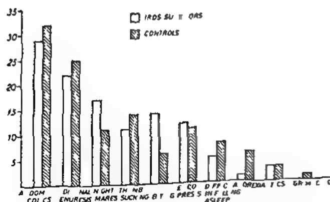


Fig 1 Percentage of psycho somatic symptoms in 65 matched pairs of IRDS survivors and controls None of the differences is significant

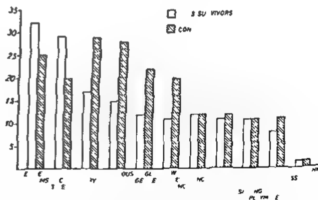


Fig 2 Percentage of behavioural characteristics in 65 matched pairs of IRDS survivors and controls None of the differences is significant

extensive epidemiologic studies from centres in Sweden (18) and Great Britain (31) have demonstrated that modern neonatal intensive care as a whole i e including optimum nutrition and rigorous correction of metabolic and respiratory disturbances has given favourable gains in surviving and undamaged babies (18) The encouraging follow up results from more studies of ventilated IRDS survivors support this view (7 29 35) with the reservation that the series up till now have been of limited size (7 20 22 24 33 35) or uncontrolled (20 22 24 29 33)

The purpose of this study has been

1 to give a picture of the physical and mental development in a series of ventilated IRDS survivors at preschool and early school age

2 to evaluate whether these children differed negatively from a series of children of the same age matched among others with respect to gestational age

3 to search for relationships between neonatal and therapeutical characteristics on one side and possible abnormalities on the other with the intention of defining prognosticly valuable risk factors

The early motor development and perhaps the early speech development were retarded in this series of IRDS survivors but at the time of the follow up it was with the exception of a single DDST subtest no longer possible to find statistically significant differences in motor or mental abilities and functioning This course of development parallels the one described by Fisch et al (13) in a series of mostly unventilated IRDS survivors suggesting that infants surviving even severe IRDS are capable to a certain extent of outgrowing an early cerebral depression The results of developmental and intelligence tests in preschool age must be interpreted with caution (38) Yet it is worth noticing that 64 out of 76 IRDS survivors judged by the DDST or Wechsler s intelligence tests could be classified as normal As mentioned by more authors (7 24) a final psychological diagnosis with regard to behavioural difficulties can hardly be made until after some years school attendance Still we find the result of the interviews concerning emotional status most encouraging Although most had one or more symptoms only few had significant problems The IRDS survivors seemed to do as well as the controls in spite of a more severe neonatal disease with often longlasting separation of mother and infant

13 out of 76 IRDS survivors or 17% presented with moderate to severe handicaps against 7% of the controls The statistical comparison (Table 6) failed to give a clear answer as to whether this prevalence was higher than expected from the degree of prematurity of the IRDS survivors as the difference came close to borderline significance

Extreme prematurity was found statistically related to later development of cerebral palsy while low birth weight for gestational age related to psycho-motor and mental retardation The vulnerability of the smallest prematures has already been stressed by several authors (22 24 35) It is more surprising that IRDS survivors who were only *moderately* small for gestational age seemed at risk of developing psycho motor and mental retardation Hagberg et al (18) in a recent publication conclude that foetal deprivation of supply seems of major importance in the pathogenesis of brain damage today We find it likely that the moderate intrauterine growth retardation in our series could be a predisposing factor rendering the brain vulnerable to otherwise tolerable degrees of extrauterine stress e g nutritional deficiencies In fact this very group of infants could be demonstrated to have had a significantly lower early milk intake than the remainder Although a cause and effect relationship between hyponutrition and brain damage cannot be established in retrospect the finding is suggestive Early tube feeding and valid parenteral nutrition in case of digestive problems might prevent brain damage in some ventilated survivors especially when small for gestational age

IRDS survivors who developed cerebral palsy on an average reached more advanced stages of respiratory insufficiency before ventilation and also had a smaller milk intake in the first week than the others The difference in pH and P_{CO_2} values as well as in volumes of milk may be due to chance, however The number of pneumothoraces and tube complications and the duration of ventilator treatment was without any difference between the IRDS survivors with and without cerebral palsy Three controls suffered from cerebral palsy without mental retardation (1 paraplegia 1 hemiparesis and 1 ataxia)

Three IRDS survivors developed *hydrocephalus* 2 of whom needed shunt operations The most handicapped of these children besides the IRDS suffered from septicemia and meningitis caused by E coli and faecal streptococci during the first week of life No controls suffered from hydrocephalus

One tetraplegic child also suffered from bilateral productive *retrolental fibroplasia* Two otherwise normal IRDS survivors had slight cicatricial *retrolental fibroplasias In* both infants high Pa_{O_2} values had been registered during ventilator treatment (peak values 31 9 and 53 3 kPa) One control was blind in one eye because of retrolental fibroplasia One unmatched IRDS survivor had an excessive myopia and strabismic amblyopia whereas one control suffered from uncomplicated myopia Furthermore 5 IRDS survivors and 4 controls presented with uncomplicated squints

Hearing loss greater than 20 dB was found in 9 IRDS survivors Three of these (one unmatched) needed hearing aid one being the above mentioned child with neonatal meningitis and hydrocephalus and another having a clear familiar etiology of otoneurological deafness The third who weighed 1 270 g at birth had both hyperbilirubinaemia (peak value= 284 μmol/l) and intensive treatment with kanamycin and gentamycin for 20 days necessitated by persistent pneumonias The other 6 IRDS survivors had either monolateral or moderate bilateral hearing losses below 40 dB Eight controls had hearing losses above 20 dB 2 of whom needed hearing aid

Two tetraplegic and one hydrocephalic child suffered from *epilepsy* Furthermore 3 IRDS survivors had had afebrile convulsions but only on a single occasion No controls had had afebrile convulsions Six IRDS survivors and 6 controls had *febrile convulsions* Dysphasic language difficulties were found in 3 IRDS survivors and 2 controls

Some of the neurological findings seem of minimal importance (i e uncomplicated squints single episodes of afebrile convulsions febrile convulsions and moderate or monolateral hearing losses) Abnormalities thought to be of at least some significance are listed in Table 6 together with developmental intellectual and emotional abnormalities *As shown one or more* abnormalities were demonstrable in 21 out of 68 matched IRDS survivors 11 of whom are thought to *have moderate to severe handicaps* (i e cerebral palsy hydrocephalus epilepsy dysphasia oligophrenia bilateral retrolental fibroplasia and bilateral hearing loss >*80 dB* singly or in combination

When 2 unmatched IRDS survivors one with excessive myopia and one with bilateral hearing loss are added the overall prevalence of moderately to severely handicapped IRDS survivors equals 17% For comparison abnormalities were found in 11 out of 68 controls 5 of whom were more than slightly handicapped (Table 6) The difference is not significant

DISCUSSION

The relationship between prematurity and physical as well as mental abnormalities is well documented (6 39) The risk of developing handicaps may be increased when IRDS supervenes (13 14 40) It is therefore understandable that critical voices have warned that mechanical ventilation of these critically ill children might result in a raised number of brain damaged survivors On the other hand

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Hof & Weisser (22) found a significant relation between the degree of respiratory insufficiency prior to ventilator treatment and developmental outcome. We also found more deranged P_{CO_2} and pH values in IRDS survivors who developed cerebral palsy as well as in IRDS survivors classified as psychomotorically abnormal and mentally retarded. Our differences did not reach statistical significance however, possibly due to the relatively small number of children in the groups. The expectant attitude to ventilatory treatment (32) may involve a risk. The indication for treatment should be revised concurrently with the advances in ventilatory technique.

Mechanical ventilation of neonates is a complicated and demanding procedure. When used by an adequately equipped and experienced staff we consider it a promising adjunct in the therapy of infants with idiopathic respiratory distress syndrome.

ACKNOWLEDGEMENTS

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Hof & Weisser (22) found a significant relation between the degree of respiratory insufficiency prior to ventilator treatment and developmental outcome We also found more deranged P_{CO_2} and pH values in IRDS survivors who developed cerebral palsy as well as in IRDS survivors classified as psychomotorically abnormal and mentally retarded Our differences did not reach statistical significance however possibly due to the relatively small number of children in the groups The expectant attitude to ventilatory treatment (32) may involve a risk The indication for treatment should be revised concurrently with the advances in ventilatory technique

Mechanical ventilation of neonates is a complicated and demanding procedure When used by an adequately equipped and experienced staff we consider it a promising adjunct in the therapy of infants with idiopathic respiratory distress syndrome

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LONG TERM PROGNOSIS OF INFANTS WITH SEVERE IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

II Cardio-pulmonary outcome

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ABSTRACT Kamper J (Department of Neonatology Rigshospitalet University of Copenhagen Denmark) Long term prognosis of severe idiopathic respiratory distress syndrome II Cardiopulmonary outcome Acta Paediatr Scand 67 71 1978.—75 out of 77 children surviving IRDS with the aid of intermittent positive pressure ventilation have been followed up by age 2 6–7 6 years together with 68 matched controls The morbidity of lower respiratory tract illnesses was significantly higher in IRDS survivors than in controls affecting a total of 48% half of whom were admitted to hospital on at least one occasion Only 3 IRDS survivors had pneumonias beyond their third year however One child suffered from a moderate stridor due to a partial laryngeal stenosis and one from some dyspnoea in function caused by broncho-pulmonary dysplasia Thoracic X ray changes were found significantly more often and more marked in IRDS survivors but on the whole the changes were discrete Neither the occurrence of pneumonia nor X ray changes in the IRDS survivors were statistically relatable to a number of neonatal or therapeutical characteristics Measurements of heart volume respiratory frequency oxygen saturation and acid–base values did not differ between the groups Ventilated IRDS survivors even with some degree of radiographic demonstrable residua thus seem to have a good long term prognosis with regard to lung function irrespective of a preliminary high morbidity of lower respiratory tract illnesses

KEY WORDS Newborn infants respiratory distress syndrome artificial respiration radiography

Modern ventilatory assistance has undoubtedly improved survival for newborn infants suffering from idiopathic respiratory distress syndrome (IRDS) but it has become clear that some survivors may develop pulmonary sequelae (15 20 21) Reports concerning later respiratory morbidity in IRDS survivors have not shown a uniform picture the incidences of respiratory illnesses ranging from normal (12) to significantly higher than normal (3 16 17)

In this report are presented the results of a follow up study in 75 children who were all treated neonatally with a positive pressure ventilator for IRDS and a group of children matched to control a number of factors known to influence the early respiratory morbidity

MATERIALS AND METHODS

Early treatment

From April 1966 to August 1971 77 out of 199 infants survived treatment with intermittent positive pressure ventilation (IPPV) for severe IRDS Diagnostic criteria indications for ventilator treatment technique method and early and late survival rates have been published in former and recent reports (10) Therapeutical parameters are indicated in Table 1

Follow up procedure

One IRDS survivor who had been adopted was excluded The remaining 76 were invited to have a follow-up examination at Rigshospitalet together with a group of 70

Table 1 *Age at beginning and duration of ventilator and oxygen treatment in IRDS survivors (h)*

	n	$\bar{x}$	Range
Age at ventilator treatment	75	35	7–10.
Duration of ventilator treatment	75	144	10–995
Duration of inspired oxygen conc =70–100 %	70	211	1–872
Duration of inspired oxygen conc =21–100%	70	425	131–1 477

^a^ 5 infants excluded because of insufficient registration

children selected as paired controls Details concerning criteria are given elsewhere (10) Three children 1 IRDS survivor and 2 controls were lost to this part of the follow up Hence the report deals with 7 unmatched and 68 matched IRDS survivors and controls Average birth weight gestational age (10) and socio-economic characteristics (Table 4) (14) for the IRDS survivors and controls differed little and insignificantly A comparison between the quality and size of the habitations of the 2 groups showed no difference (Table 2) more than half of both groups living in flats or houses with central heating water and electricity installed (=modern) and at least one room per family member (=roomy)

Chest X rays 129 children all examined at the follow up clinic had chest X-rays taken in the antero-posterior and lateral projections Pulmonary changes were assessed according to the method published by Chrispin & Norman in 1974 (5) Both lung fields in this study have been assessed as a whole without the originally proposed subdivision Changes in chest configuration and lung fields were graded blindly by two radiologists (H S & L J) as not present (0 points) present but not marked (1 point) and present and marked (2 points) Finally all points were added leaving a radiographic score of 0–14 points

Cardiac volume was measured and calculated according to the method published by Liljestrand et al (13) The shape of the heart was evaluated with special attention to signs of right heart and pulmonary trunk enlargements

Blood samples Blood for acid–base balance and oxygen saturation was drawn in 128 cases from capillary punctures in a hyperaemised ear lobe with the child sitting at rest breathing atmospheric air The acid–base analyses were performed within minutes using the Astrup-technique (22) Oxygen saturations were made at the same time but failed on 5 occasions

Statistics

Statistical computations were done employing the χ^2 test with Yate s correction factor and Student's *t* test Comparisons of therapeutical data indicated in Table 1 were also made with the Wilcoxon test for independent variables as these parameters are not normally distributed

In comparison of IRDS survivors and controls only matching pairs of the two series have been used When ever data were lacking in one of a pair both children were omitted The actual number of match-pairs is indicated in tables and text

RESULTS

Laryngeal stenosis necessitated tracheostomy in 2 out of 75 IRDS survivors at the age of 3 and 9 months The tracheostomies were successfully closed 10 and 19 months later but one of the children was still somewhat handicapped in physical effort by stridor and by a tendency to lower airway infections The average duration of ventilator treatment—and intubation—for these two children was 112 hours which is well below the average for the whole group of IRDS survivors (144 hours)

Upper airway infections resulting in medical treatment occurred with almost the same frequency among matched IRDS survivors and controls as indicated in Table 3 The single most frequent disease was middle ear infection Two children in each group still suffered from recurrent infections

Lower airway infections Among the 75 IRDS survivors 48% suffered from pneumonia or bronchiolitis (characterized by wheezing expiratory dyspnoea cough and fever) and 25% had been admitted to hospital at least once for that reason Only 3 survivors had lower respiratory tract infections after the age of 3 years however one boy with tetraplegia and insufficient coughing reflex another boy with laryngeal stenosis and one girl who had the middle lobe of the right lung resected in

Table 2 *Distribution according to quality and size of habitations in matched IRDS survivors and controls*^a^

	Ventilated	Controls
Old or not roomy	31	30
Modern and roomy	37	38
Total	68	68

$\chi^2=0$ d.f. $=1$ $p=1.0$

Table 3 *Respiratory infections resulting in medical treatment in 68 IRDS survivors and controls*

	Ventilated	Controls	p
Upper airway infections one	[illegible]	49	N s
Upper airway infections 2 or more	43	41	N s
Bronchiolitis	7	3	N s
Pneumonia one	[illegible]	13	0 008
Pneumonia 2 or more	[illegible]	6	N s
Pneumonia causing hospitalization	[illegible]	5	0 006

Not significant

her second year of life because of an otherwise intractable infection

Comparing 68 matched IRDS survivors and controls (Table 3) bronchiolitis and pneumonia occurred about twice as often in the IRDS survivors While the difference in incidence of pneumonia is statistically significant ($p<0\ 01$) figures for bronchiolitis and more than one pneumonia were too small to make the difference significant 3–4 times as many IRDS survivors suffered from pneumonia severe enough to cause hospitalisation ($p<0\ 01$) As shown in Table 4 significantly more IRDS survivors belonging to the upper social classes 3–6 went to hospital for pneumonia ($p<0\ 05$) This indicates that other than socio-economic factors may have predisposed the IRDS survivors to severe pneumonias Therefore IRDS survivors who developed pneumonia causing hospitalisation were compared with IRDS survivors who did not with regard to a number of neonatal and therapeutical parameters While mean gestational age and birth weight were quite similar in the two groups a tendency was found for the former to need ventilator therapy earlier (29 h versus 37 h) and for a longer time (184 h versus 128 h) than the latter The former also needed raised inspiratory oxygen [illegible] any concentration for a longer period (21–100% 464 h versus 414 70–100% 235 h versus 204) No differences reached statistical significance however

Radiological abnormalities

The prevalence of radiological abnormalities in IRDS survivors and controls is illustrated in Fig 1 Abnormalities were found in 21 out of 57 matched IRDS survivors (37%) and in 9 out of the controls (16%) The difference in number of children with line shadows also reached significance ($p<0\ 05$) The abnormalities were also more marked in the IRDS survivors as expressed by a significantly higher mean radiographic score=0 58 against 0 18 in the controls ($p<0\ 01$) On the other hand [illegible] mean score of 0 58 out of 14 possible clearly demonstrates that the radiological changes were rather discrete even in the IRDS survivors Only one IRDS survivor a boy of 4 years had X ray changes compatible with broncho pulmonary dysplasia At the follow up he presented a marked emphysema without heart enlargement His only symptom was dyspnoea at function and oxygen saturation as well as acid–base values were within normal limits

Within the group of IRDS survivors 23 children had abnormal X rays When compared to 41 IRDS survivors with normal X rays with regard to birth weight gestational age therapeutical parameters (Table 1) and incidence of pneumonia no differences reached statistical significance

As shown in Table 5 mean values for respiratory frequency oxygen saturation and acid–base parameters were rather similar in matched IRDS survivors and controls as was

Table 4 *Social class distribution in matched IRDS survivors and controls*

Number of children with one or more pneumonias causing hospitalization indicated in parentheses

Social class	Ventilated	Controls
3–4	9 (1)	9 (0)
5–6	[illegible]7 (7)	[illegible]4 (1)
7	14 (4)	16 ([illegible])
8	[illegible] (6)	19 (2)
Total	68 (18)	68 (5)

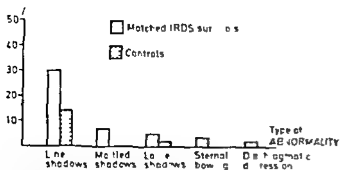


Fig 1 *Prevalence of X ray abnormalities in 57 matched* pairs of IRDS survivors and controls

the case in IRDS survivors with and without X ray abnormalities

No children had clinical or radiological evidence of heart disease The small difference in mean heart volume between the groups is not significant

DISCUSSION

One prerequisite of prolonged ventilation of neonates with intermittent positive pressure has been the introduction of plastic tubes which permit oro and naso-tracheal intubation for weeks Although autopsy (9) and follow up studies (4) in intubated IRDS infants have shown a high proportion of both acute and residual laryngeal lesions the occurrence of clinically significant *laryngeal stenosis* needing tracheostomy has generally been low Hof & Weisser (8) in an early studie reported laryngeal stenoses among 38% of their children however These authors stated that this complication could later be prevented by administering colistine prophylactically against pseudomonas tracheites and by frequent changing of the tracheal tubes Our patients did not have their tubes replaced unless they became blocked suggesting that frequent tube replacement is unnecessary

The incidence of *pneumonia and bronchiolitis* was high and significantly higher than in the control group It was also higher than in 21 infants surviving ventilation for other reasons than IRDS during the same period (11) This high incidence of lower respiratory tract infections has been documented by Outerbridge et al (16 17) among others who has most recently called attention to the problem The question now arises whether the predisposition to lower respiratory tract infections is caused by intrinsic factors in the regenerating IRDS lung (2) or rather by iatrogenic factors related to therapy e g mechanical ventilation or the usage of oxygen in high concentrations for several days Zachau Christiansen (25) has provided evidence that IRDS survivors from the preventilator era also had a higher morbidity of lower respiratory tract infections in comparison to infants belonging to the same birth weight groups and socio-economic classes This author thus reported an average incidence of 32% during the first year of life Our higher incidence—48%—may be explained by our longer time of observation but also by the severer degree of IRDS in infants surviving with the aid of a

Table 5 *Respiratory frequency oxygen saturation acid base status and heart volume in matched IRDS survivors and controls*

	No of comparisons	Ventilated $\bar{x}$	Ventilated S D	Ventilated (Range)	Controls $\bar{x}$	Controls S D	Controls (Range)
Breath per min	49	21 9	5 2	(12–34)	23 1	4 6	(16–32)
Oxygen saturation	52	0 96	0 017	(0 92–1 00)	0 96	0 024	(0 89–1 00)
P_{CO_2} (kPa)	55	4 39	0 32	(3 73–5 07)	4 39	0 33	(3 60–5 33)
pH	55	7 43	0 02	(7 39–7 47)	7 43	0 02	(7 40–7 47)
Standard bicarbonate (mmol/l)	55	23 0	1 3	(20 4–26 2)	23 0	1 5	(19 8–26 5)
Heart volume (ml/m² body surface)	57	284	38 3	(206–370)	275	39 8	(183–373)

ventilator Like Outerbridge et al (16 17) we were not able to find statistically significant relations between the predisposition to pneumonia and any therapeutical parameter These facts taken together support the hypothesis that the predisposition to lower respiratory tract infections in IRDS survivors is mainly a consequence of IRDS itself (17)

Infants with severe IRDS who survive with the aid of ventilator therapy may develop varying degrees of *septal and peribronchiolar fibrosis* (20 21) In the most extreme forms the interstitial fibroplasia is associated with emphysema and sometimes cor pulmonale in which case it is usually fatal This complication termed chronic or stage IV *bronchopulmonary dysplasia* was originally described by Northway et al (15) who found the condition associated with the use of *both IPPV and raised inspiratory oxygen* in concentrations of at least 80% (high oxygen) for 150 hours Although the subject has been dealt with by several authors (1 6 18 19) and oxygen toxicity most often implicated no fully satisfactory explanation has been given as to the cause In fact it seems to be multifactorious in origin (23) It is remarkable that only one long term survivor in our series of 75 children developed chronic bronchopulmonary dysplasia in spite of a mean duration of IPPV and high oxygen (at least 70%) to 144 and 211 hours respectively This indicates that procedures connected with IPPV such as humidification mucus drainage infection prophylaxis and perhaps ventilatory pattern (1) may have been of importance in minimising the incidence of this condition

Bryan et al (3) have performed serial measurements of pulmonary function in IRDS survivors during the first year of life While non ventilated survivors returned to normal function by age two to four months the more severely affected *ventilated infants* did not recover totally during the first year According to Harrod et al (7) marked X ray changes including emphysema as well as gas exchange abnormalities may persist in such children by age one to five years In contrast the present study has shown that a large majority of ventilated IRDS survivors at the time of follow up had either normal X rays or grade I line infiltrates suggesting minimal interstitial fibrosis (21) which did not affect pulmonary gas exchange capacity These findings support the view (12 24) that infants with IRDS surviving with intermittent positive pressure ventilation usually have a good long term prognosis with regard to lung function It must be emphasized however that the mean age in the present study group was only 4 8 years and that the encouraging results have to be confirmed by more extensive pulmonary function studies when the children have grown older

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EFFICACY OF ZOSTER IMMUNOGLOBULIN IN PROPHYLAXIS OF VARICELLA IN HIGH RISK PATIENTS

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ABSTRACT Winsnes R (Vaccine Department National Institute of Public Health Oslo Norway) Efficacy of zoster immunoglobulin in prophylaxis of varicella in high risk patients Acta Paediatr Scand 67 77 1978 —During a period of 2 1/2 years 190 children have been prophylactically treated with zoster immunoglobulin in Norway Information on 130 (68 %) of these children was obtained This group included patients suffering from leukemia lymphoma and other malignant disorders and patients with autoimmune diseases with impaired immune mechanisms or with for other reasons increased frequency of infections as well as premature and weak newborns and infants born to mothers who developed varicella during the last 4 days before delivery Of the patients who received zoster immunoglobulin within 3 days after exposure 2 9% developed weak symptoms of varicella When zoster immunoglobulin was administered 4–5 days after exposure 37 5% contracted varicella and when given more than 5 days after exposure 50% developed the disease The 9 neonates with possible intrauterine exposure are not included in these figures

KEY WORDS Zoster immunoglobulin varicella passive immunity immune serums

Varicella is a highly contagious primary infection caused by varicella zoster virus (VZV) (16) The course of the disease varies from a mild cutaneous infection to severe visceral involvement Although varicella is usually a benign childhood disease there are illnesses that seem to predispose to a higher risk of severe and potentially fatal infection These high risk conditions include leukemia malignant lymphoma (9 28) immunodeficiency syndromes (21) and treatment with immunosuppressive medications (10 13 19) Another high risk group includes children born to mothers who develop varicella during the last 4 days before delivery Reviewing 13 cases of the latter category Meyers (25) reported a 31% case fatality ratio When combining with the 17% attack rate 5% of term infants born live died of varicella infection

As no documented reliable mode of therapy for varicella in patients at high risk exists the patients in this study received prophylactic treatment with zoster immunoglobulin (ZIG) Treatment with ZIG has been reported to be beneficial in prevention of varicella in normal children (3) and in immunocompromized patients (5 15 23 26) In a comparative study with ordinary gamma globulin zoster immune plasma significantly reduced the incidence of clinical varicella and attenuated the severity of its course (12)

PATIENTS AND METHODS

Patients

During the period june 1974–february 1977 190 children received prophylactic treatment with ZIG Information on clinical course was obtained for 130 of these children Only 6 of the 130 children were above 10 years of age The patients may be divided into the following groups and subgroups

I Lympho- and myeloproliferative diseases Leukemia

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Table 2 *Course of varicella compared to interval between exposure and zoster immunoglobulin administration indication for prophylactic treatment and medication*

Patient	Sex	Age	Interval exposure-ZIG administration (days)	Indication	Medication	Course of varicella
K J Å	M	–	<3	Leukemia	Cyt [b] cort	Mild[d]
H L	F	6 years	<3	Leukemia	Cort	Very mild
V V	F	6 years	3	Lupus erythematosus disseminatus	Gammaglobulin	Very mild
S K	M	–	4–5	Leukemia	Cyt cort	Mild
B H	M	11 years	4–5	Leukemia	No	Mild
Å W	F	9 years	4–5	Ulcerous colitis	Cort	Very mild
G A P	M	–	>5	Leukemia	Cort	Mild
S H	M	–	>5	Leukemia	Cyt	Mild
T S	M	7 years	>5	Leukemia	Cyt	Mild
Baby T	M	35 days	>5	Weak premature	No	Very mild

Zoster immunoglobulin [b] Cytostatica. Corticosteroides
[d] Below 20 pox or characterized as milder than other family members
Fever without exanthema or a few pox without fever

oped herpes zoster at the end of pregnancy All the 9 neonates received ZIG Only 2 of the children whose mothers developed varicella within 4 days before delivery developed the disease The course was very mild in these 2 infants with appearance of a few vesicles only

Interval between exposure and administration of ZIG is compared to attack rate of varicella in Table 3 The intervals given are based on data recorded or on calculation of a minimal incubation period of 14 days (16) Since there are various theoretical considerations concerning the time of possible exposure of the foetus (16) the neonates who may have been exposed *in utero* are not included in Table 2 and 3

Of 121 evaluated patients 105 received ZIG

Table 3 *Interval between exposure and zoster immunoglobulin administration in relation to attack rate of varicella*

Interval exposure ZIG [redacted] ministration	No of patients	Varicella cases	Attack rate (%)
≤3 days	105	3	9
4–5 days	8	3	37 5
>5 days	8	4	50 0

Zoster immunoglobulin

within 3 days after exposure Of these patients 33 were given ZIG with a CFA titer of 2 560 and 72 were given ZIG with a CFA titer of 1 500 None of the 33 but 3 of the 72 patients developed varicella

Serum antibody

Seven children were excluded from this study because pretreatment serum specimens contained CFA against VZV None of these developed varicella

Pre treatment serum specimens were received from 44 of the 130 evaluated persons CFA titers were <2 Nuclear and cytoplasmic fluorescence was not seen in any of these specimens by indirect fluorescence procedure

Serum specimens collected 6 to 8 weeks after administration of ZIG were received from 25 of the 44 and from another 2 persons CFA titer against VZV in serum samples from patients who developed clinical manifestations of varicella was <4 (8 patients) and 24 (1 patient)

In corresponding serum samples from 18 patients with no history of clinical varicella after ZIG administration CFA titer against VZV was <4 (12 patients) 4 (4 patients) [redacted] (1 patient) and 16 (1 patient) Presence of antibodies against VZV in serum samples was confirmed by FA tests

(52 patients) lymphosarcoma (2 patients) Hodgkin s disease (2 patients) myeloproliferative syndrome (1 pa tient)

2 Other malignancy Osteosarcoma (1 patient) Wilm s tumour (1 patient)

3 Autoimmune diseases Aplastic anemia (3 patients) lupus erythematosus disseminatus (1 patient)

4 Infants born to mothers who developed varicella or herpes zoster at the end of pregnancy Infants born to mothers who developed varicella within 4 days before delivery (5 patients) infants whose mothers developed varicella 7 to 14 days before delivery (2 patients) in fants whose mothers developed herpes zoster at the end of pregnancy (2 patients)

5 Weak newborns Premature infants born to mothers with no clinical history of varicella (15 patients) term infants whose mothers had no history of clinical varicella and no detectable complement fixing antibodies (CFA) against VZV (23 patients)

6 Patients with impaired immune mechanisms or with for other reasons increased frequency of infections Hemorrhagic diathesis (Factor XII deficit) (1 patient) mucocutaneous syndrome (1 patient) ulcerous colitis (1 patient) nephrosis (2 patients) Down s syndrome (1 pa tient) oesophageal atresia (1 patient) sepsis (1 patient) encephalitis (1 patient) gastrochisis operata (1 patient) children given vaccinia vaccine (3 patients) steroid therapy and asthma (2 patients) steroid therapy and rheumatic diseases (3 patients) and 2 patients who could not be removed during a varicella outbreak in the hospital ward

Treatment

ZIG was given intramuscularly in doses of 0 15 ml/kg body weight with a minimum of 1 ml and a maximum of 5 ml The administration of ZIG was recommended to take place within 3 days after exposure

Preparation of ZIG

Sera for production of ZIG were obtained from healthy convalescents 14 to 28 days after the onset of zosteriform or varicelliform rash and selected for preparation on the basis of CFA titer levels Only plasma with absence of hepatitis B antigen (HB Ag) evaluated by radioimmuno-assay were used ZIG was prepared essentially as de scribed by Falksveden (8) The final solution contained 16% of IgG stabilized with glycine The CFA titer against VZV of the preparations used in this study was either 2 560 or 1 500 As another measure of potency of the IgG molecules content of anti rubella virus antibodies was determined by hemagglutination inhibition test (20) The immunoglobulin preparations met the requirements for Human Normal Immunoglobulin according to European Pharmacopoeia 1971 (7) with the exception that the plasma was collected from 37 to [illegible] donors 2 to 4 weeks after outbreak of zoster or varicella

In vitro tests

Serum specimens collected before and 6 to 8 weeks after prophylactic treatment were requested The specimens were analyzed for content of CFA and fluorescent anti bodies (FA) against VZV using serum dilutions of 1 4

Table 1 *Clinical attack rate of varicella in patients treated with zoster immunoglobulin by exposure status*

Type of exposure	No of pa tients	Per cent of total	Vari cella cases	Attack rate (%)
Hospital	95	73 1	5	5 3
Household	12	9 2	4	33 3
Playmate	10	7 7	1	10 0
Maternal vari cella zoster	9	6 9	2	[illegible]
Unknown or other	4	3 1		
Total	*130*	*100*	*12*	*9 [illegible]*

A micromethod was used for determination of CFA titer (34) using a barbital buffer with calcium and magnesium Anticomplementary activity was removed (32) using 0 4 ml undiluted guinea pig serum to [illegible] 1 ml of 16% ZIG solution After incubation in water at 37 C for 30 mi excess complement was inactivated VZV antigen negative control antigen and a control serum from Behringwerke AG was used As in vitro antigenic similarities between herpes simplex virus and VZV have been de scribed (33) the positive serum specimens were checked for possible serologic cross reacting antibodies Herpes simplex virus type 1 antigen was purchased from the same commercial source

FA tests (18) were carried out for investigation of susceptibility to VZ infection Human diploid lung cells infected with VZV for preparation of slides and fluo resceinisothiocyanate-conjugated antiserum to human immunoglobulin were obtained from Statens Bakteriolo-giska Laboratorium Stockholm Sweden The conjugate contained anti IgG antibodies 1 7 mg/ml Molar ratio of fluoresceinisothiocyanate to IgG was 2 9 A dilution of 1 15 was used A Zeiss standard 14 microscope was used with epi fluorescence condenser IV FL standard filter combinations for fluoresceinisothiocyanate con jugates and high pressure mercury lamp HBO 50 W

RESULTS

Clinical course

Exposure status and clinical attack rate of the 130 evaluated patients are given in Table 1 Of the 130 patients 12 developed a mild varicella or only a transient rise in temperature Data on 10 of the patients who developed varicella are given in Table 2

Seven women developed varicella before delivery 5 within the last 4 days and 2 within the last 2 weeks In addition 2 women devel

higher CFA titer than the others None of the 33 patients developed varicella The observation that high risk children were not protected against varicella as successfully as normal children (2 3) suggests the need for high potency preparations

Systemic varicella and fatalities from the infection may occur in childhood (30) adulthood (34) as well as pregnancy (1 11 27) in patients with apparently normal immune defence Congenital anomalies are rare but may be serious consequences of varicella zoster infection early in pregnancy whether the infection occurs in the form of varicella or herpes zoster (36) As the problem continually has been to collect sufficient amounts of sera with high CFA titer against VZV, and most adults who cannot recall having varicella are probably immune (29) the use of ZIG for adults cannot receive high priority at present Also other countries have difficulties in providing enough plasma for ZIG production (4) Efforts are made for increasing the supply of VZ plasma in Norway However ZIG still remains an investigational medication in short supply (26)

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DISCUSSION

In the present study susceptibility of patients to varicella infection has been investigated using both FA and CFA assays Efficacy of prophylactic treatment with ZIG may not be firmly documented using these serologic techniques only but must await the development of an array of techniques to measure various parameters of the immune response Previous varicella will not always be detected by employing CFA test (14) Recently FA assays claimed to be specific and more sensitive than the CFA technique have been used (18 35)

Serum samples were obtained from 44 of the 130 (34%) evaluated patients It may be objected that the susceptibility of the remaining 86 patients is unknown However clinical signs of varicella had not been observed The average age of these patients was 5 years and it is unlikely that a clinical attack would have been overlooked by the parents

An average attack rate of 2 9% for patients who received ZIG within 3 days after exposure is the lowest attack rate reported to date for high risk patients Presumably the large number of hospitalized children who received prophylactic treatment with ZIG (Table 1) has influenced this figure An attack rate of 8% has been reported for 63 high risk patients with hospital exposure (5) In the present as well as in other studies (5) the attack rate is highest for patients with household exposure

From Table 3 it is evident that initiation of prophylactic treatment ought to take place within 3 days after exposure in order to prevent clinical disease Administration of ZIG later than 5 days after exposure may not affect the attack rate but the course of varicella may be attenuated (Table 2) An attack rate of 50% may be consistent with the generally accepted attack rate of 75% among exposed susceptibles (16) as the attack rate is relatively low after hospital exposure Among mentally retarded children in an institution the attack rate was 60% when based on clinical observations alone and between 75 and 82% when based on clinical and serological observations (14)

If no information was obtained on the exact time of exposure or the given date was inconsistent with the date of outbreak of varicella a minimal incubation period of 14 days was calculated (14 16) A minimal incubation period of 11 days has been reported (17) Reports by others (2 15) indicate that administration of ZIG may result in a prolonged incubation period An estimated period of latency of 14 days seems to be reasonable among these patients The infants with possible congenital exposure are excluded from Table 2 and 3 as the time of exposure cannot be established (16)

It may be argued that totally protective doses of ZIG should be administered if possible The doses used in Norway correspond to those used by others (6) In the present study no clinical manifestations of varicella appeared in 97 1% of patients who received ZIG within 3 days after exposure Seroconversion may have taken place in 6 (33%) of 18 patients without clinical varicella from whom a second serum sample was obtained It is unlikely that presence of antibodies against VZV in the second serum samples is due to the administered ZIG as reports by others (22) indicate that ZIG is undetectable [illegible] weeks after injection Low antibody levels after clinical varicella may be partly due to the underlying disease and to the administration of immunosuppressive medication and specific antibody

It is an open question if patients with impaired defence mechanisms will be adequately immunized to sustain a subsequent VZ infection In our experience (31 37) varicella may sometimes be succeeded by a fatal generalized herpes zoster in patients with leukemia No information of herpes zoster in the prophylactically treated patients has been received to date

In the present study 33 of 105 patients to whom ZIG was administered within 3 days after exposure received a preparation with a

higher CFA titer than the others None of the 33 patients developed varicella The observation that high risk children were not protected against varicella as successfully as normal children (2 3) suggests the need for high potency preparations

Systemic varicella and fatalities from the infection may occur in childhood (30) adulthood (34) as well as pregnancy (1 11 27) in patients with apparently normal immune defence Congenital anomalies are rare but may be serious consequences of varicella zoster infection early in pregnancy whether the infection occurs in the form of varicella or herpes zoster (36) As the problem continually has been to collect sufficient amounts of sera with high CFA titer against VZV and most adults who cannot recall having varicella are probably immune (29) the use of ZIG for adults cannot receive high priority at present Also other countries have difficulties in providing enough plasma for ZIG production (4) Efforts are made for increasing the supply of VZ plasma in Norway However ZIG still remains an investigational medication in short supply (26)

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24 Lennette E H Complement fixation test In E H

DISCUSSION

In the present study susceptibility of patients to varicella infection has been investigated using both FA and CFA assays Efficacy of prophylactic treatment with ZIG may not be firmly documented using these serologic techniques only but must await the development of an array of techniques to measure various parameters of the immune response Previous varicella will not always be detected by employing CFA test (14) Recently FA assays claimed to be specific and more sensitive than the CFA technique have been used (18 35)

Serum samples were obtained from 44 of the 130 (34%) evaluated patients It may be objected that the susceptibility of the remaining 86 patients is unknown However clinical signs of varicella had not been observed The average age of these patients was 5 years and it is unlikely that a clinical attack would have been overlooked by the parents

An average attack rate of 2 9% for patients who received ZIG within 3 days after exposure is the lowest attack rate reported to date for high risk patients Presumably the large number of hospitalized children who received prophylactic treatment with ZIG (Table 1) has influenced this figure An attack rate of 8% has been reported for 63 high risk patients with hospital exposure (5) In the present as well as in other studies (5) the attack rate is highest for patients with household exposure

From Table 3 it is evident that initiation of prophylactic treatment ought to take place within 3 days after exposure in order to prevent clinical disease Administration of ZIG later than 5 days after exposure may not affect the attack rate but the course of varicella may be attenuated (Table 2) An attack rate of 50% may be consistent with the generally accepted attack rate of 75% among exposed susceptibles (16) as the attack rate ■ relatively low after hospital exposure Among mentally retarded children in an institution the attack rate was 60% when based on clinical observations alone and between 75 and 82% when based on clinical and serological obser\ at
(14)

If no information was obtained on the e
time of exposure or the given date was
consistent with the date of outbreak of \
cella a minimal incubation period of 14 (
was calculated (14 16) A minimal incuba
period of 11 days has been reported (17)
ports by others (2 15) indicate that adminis
tion of ZIG may result in ■ prolonged inc
tion period An estimated period of latenc
14 days seems to be reasonable among t
patients The infants with possible conge
exposure are excluded from Table 2 and :
the time of exposure cannot be establi
(16)

It may be argued that totally prote
doses of ZIG should be administered if
sible The doses used in Norway corres
to those used by others (6) In the pr
study no clinical manifestations of van
appeared in 97 1% of patients who rec
ZIG within 3 days after exposure Sero
version may have taken place in 6 (33%) (
patients without clinical varicella from w
■ second serum sample was obtained
unlikely that presence of antibodies ag
VZV in the second serum samples is du
the administered ZIG as reports by ot
(22) indicate that ZIG is undetectable 8 w
after injection Low antibody levels after (
cal varicella may be partly due to the ur
lying disease and to the administration ■
munosuppressive medication and specific
body

It ■ an open question if patients with
paired defence mechanisms will be adequa
immunized to sustain a subsequent VZ i
tion In our experience (31 37) varicella
sometimes be succeeded by ■ fatal genera
herpes zoster in patients with leukemia N
formation of herpes zoster ■ the prophyl
cally treated patients has been receive
date

In the present study 33 of 105 patient
whom ZIG was administered within 3
after exposure received ■ preparation w

higher CFA titer than the others None of the 33 patients developed varicella The observation that high risk children were not protected against varicella as successfully as normal children (2 3) suggests the need for high potency preparations

Systemic varicella and fatalities from the infection may occur in childhood (30) adulthood (34) as well as pregnancy (1 11 27) in patients with apparently normal immune defence Congenital anomalies are rare but may be serious consequences of varicella zoster infection early in pregnancy whether the infection occurs in the form of varicella or herpes zoster (36) As the problem continually has been to collect sufficient amounts of sera with high CFA titer against VZV and most adults who cannot recall having varicella are probably immune (29) the use of ZIG for adults cannot receive high priority at present Also other countries have difficulties in providing enough plasma for ZIG production (4) Efforts are made for increasing the supply of VZ plasma in Norway However ZIG still remains an investigational medication in short supply (26)

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FACTOR VIII ACTIVITY AND ANTIGEN IN SICK NEWBORNS WITH PATHOLOGICAL PROTEOLYSIS IN BLOOD

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ABSTRACT Henriksson P and Holmberg L (Dept of Paediatrics and the Coagulation Laboratory Malmo Allmanna Sjukhus Malmo Sweden) Factor VIII activity and antigen in sick newborns with pathological proteolysis in blood Acta Paediatr Scand 67 [illegible] 1978 —Factor VIII clotting activity (VII C) and factor VIII related antigen (VIII R AG) were determined in 12 sick newborn infants with pathological proteolysis in their circulation A marked discrepancy was noted between VIII R AG and VIII C the ratio in sick infants being on average 2 1 while no discrepancy was seen in healthy newborns The finding in the sick infants resembled a low grade plasminogen activation which was studied experimentally It is concluded that demonstration of a marked discrepancy between VIII R AG and VIII C is a useful indication of pathological proteolysis in sick newborns

KEY WORDS Factor VIII pathological proteolysis newborn

Many neonatal disorders are accompanied by a state of pathological proteolysis in plasma (5) sometimes leading to an activation of the coagulation system as well as of the fibrinolytic system If the former system is activated more strongly than the latter intravascular coagulation will ensue with the risk of impairment of the microcirculation If activation of the fibrinolytic system is predominant bleeding is more likely to occur In the treatment of a given patient it may thus be crucial to decide which of the two systems is preponderantly activated

Factor VIII clotting activity (VIII C) is defined as the activity missing in classical haemophilia A Low levels of VIII C occur both in intravascular coagulation and fibrinolysis However in a previous study (5) we did not find VIII C to be low in sick newborns with signs of pathological proteolysis As sociated with VIII C in plasma is a protein so-called factor VIII related protein (16) which can be determined immunologically (VIII R AG factor VIII related antigen) This protein resists proteolytic degradation better than VIII C (13) A discrepancy between VIII C and VIII R AG may thus indicate pathological proteolysis

The purpose of this paper was to assess the relationship between VIII C and VIII R AG in sick newborns with pathological proteolysis The molecular structure of factor VIII in such patients was examined with crossed immuno electrophoresis and the pattern compared with the effects of plasmin and thrombin on factor VIII in an experimental model

CLINICAL MATERIAL

The material consisted of 12 infants (Table 1) Four of them had IRDS ? had unspecified respiratory symptoms 3 had apnea repetens and 3 had perinatal asphyxia Six died in the neonatal period 2 from IRDS 2 from apnea repetens and 2 from asphyxia All the patients had signs of pathological proteolysis as indicated by the appearance of fibrin/fibrinogen degradation products in serum

Table 1 *Factor VIII activity and antigen in 12 sick newborns*

Diagnosis	Outcome	*n*		F VIII C (%)	F VIII R AG (%)
IRDS (Hyaline membrane disease)	Survived	2		80 138	224 146
	Died	2		94 188	198 260
Unspecified respiratory distress	Survived	2		77 79	280 172
Apnea repetens	Survived	1		70	285
	Died	2		135 58	155 225
Asphyxia	Survived	1		83	138
	Died	2		50 320	670 370
Total		12	range	50–320	138–670
			mean	114	260
			S D	75	145

Gastrointestinal malformations (extensive bowel gangrene)

The gestational ages ranged between 26 and 40 weeks (median 35 weeks) The birthweights ranged between 700 and 2910 grams (median 2 150 grams)

METHODS

Blood was obtained via an indwelling umbilical artery catheter It was collected with the silicone technique and citrated plasma was prepared as previously described (7) The plasma was frozen immediately and stored at −60°C until examined

VIII C and VIII R AG were determined in the way described by Holmberg & Nilsson (8) Plasmin was tested for any effect on factor VIII by incubating normal plasma with various amounts of plasmin Lysofibrin (Novo) was dissolved in saline 210 CTA units/ml and added to plasma to a final concentration of 1 75 17 5 or 35 CTA units/ml The results were corrected for the slight dilution of the plasma (8 5–19%) VIII C and VIII R AG were examined immediately as well as after 4 and 24 hours incubation at 37°C The antigen was examined both with Laurell s electroimmunoassay and with crossed immunoelectrophoresis (3)

Fibrin/fibrinogen degradation products (FDP) were determined with the immunochemical method of Nilehn (12) The determinations of FDP were performed on serum obtained from blood collected in tubes containing thrombin and EACA

RESULTS

The results of the clinical investigation are given in Table 1 The mean VIII C was 114% S D 75% (Normal value for full term healthy newborns is 116 6% S D 41 2%) The mean VIII R AG was 260% S D 145% (Normal value for full term healthy newborns is 120% S D 44 5%) The difference in VIII R AG between sick and healthy newborns was statistically significant ($p<0.001$)

In 7 of the 12 patients the VIII R AG/VIII C quotient was above 2 0 Three out of 6 patients who died and 4 out of the remaining 6 who survived had quotients above 2 0

In the 2 patients with IRDS and VIII R AG/

Table 2 *The effect of plasmin on factor VIII activity and antigen*

Plasmin concentration (CTA units/ ml plasma)	Incubation time (hours) at 37 C	Factor VIII Activity (%)	Factor VIII Antigen (%)
35	0	1	139
35	4	0 5	142
35	24	0 5	179
17 5	0	1	113
17 5	4	0 5	122
17 5	24	0 5	159
1 75	0	19	82
1 75	4	9	102
1 75	24	8	106
0	0	85	92
0	4	56	97
0	24	56	104
Control plasma		115	103



Fig 1 Crossed immunoelectrophoresis of normal plasma (*top*) and of normal plasma incubated with Lysofibrin 35 CTA units/ml for 24 hours (*bottom*)

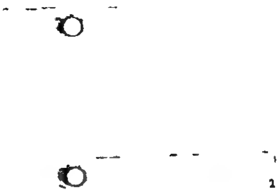


Fig 2 Crossed immunoelectrophoresis of normal plasma (*top*) and of normal serum (*bottom*)

VIII C quotients above 2 0 crossed immuno electrophoresis of plasma revealed no change in mobility of VIII R AG

The results of the experimental studies with plasmin are given in Table 2 VIII C was rapidly destroyed by plasmin in high concentrations while a low concentration resulted only in decreased activity VIII R AG was not immediately affected by the addition of large amounts of plasmin (17 5–35 CTA units/ml) but after 24 hours incubation it was increased Crossed immunoelectrophoresis of plasma samples incubated in that way revealed that the migration of VIII R AG was abnormally fast especially when the concentration of plasmin was relatively high (Fig 1) VIII R AG was not affected by plasmin in low concentrations (1 75 CTA units/ml)

The effect of thrombin on factor VIII can be demonstrated by investigating serum The amount of VIII R AG in serum was equal to or only slightly less than that in plasma The crossed immunoelectrophoresis of VIII R AG in serum (Fig 2) showed no difference in the shape or mobility of the peak compared with that in plasma

DISCUSSION

The factor VIII molecule has a dual biological function It is necessary not only for normal intrinsic plasma coagulation (VIII C) but also for normal primary haemostasis i e the formation of the primary platelet plug (von Willebrand factor activity) Experimental data (2) suggest that the molecule is made up of two components The first which has the von Willebrand factor activity is quantitatively predominant and can be measured immunologically as VIII R AG This component is fairly stable and is not consumed during coagulation The second component which has the VIII C is much smaller and is labile The molecular structure of the second component has not yet been well defined and it may perhaps not be a separate molecule (15)

The biological range of variation of VIII C as well as VIII R AG is wide Both increase in association with stress due to exercise (1 14) pregnancy and surgical operations (6) and in certain disorders it is associated with tissue break-down and repair (8) e g liver disease widespread cancer and burns In many of these diseases a discrepancy has been

noticed between VIII C and VIII R AG and explained by low grade in vivo coagulation and/or fibrinolysis (6) VIII C often decreases in these conditions (9 11)

Intravascular coagulation and fibrinolysis have been reported to occur in sick newborns (4) However, in a previous study we found normal VIII C even in very sick newborn infants with signs of pathological proteolysis (5) The present study offers an explanation for this finding In almost all of the cases VIII R AG was substantially raised and was much higher than that of VIII C In 7 of 12 patients the concentration of VIII R AG was more than twice that of VIII C Such a high ratio is not seen in normal newborn infants (7) The sick infants are in a state of stress which increases VIII R AG by mobilization of the protein from pools and probably by enhanced synthesis (1, 6) Stress such as physical exercise, increases VIII C to the same extent as VIII R AG (1 10) The fact that VIII C unlike VIII R AG is not increased in the stressed sick newborn suggests an accelerated breakdown or consumption of the clot promoting labile structures of the factor VIII molecular complex i e a pathological proteolysis Yet VIII C is usually not abnormally low since the consumption is counterbalanced by the increased mobilization and synthesis

The discrepancy between VIII C and VIII R AG may be due to the effect of thrombin (intravascular coagulation) or of plasmin (fibrinolysis) The extreme effect of thrombin is seen in serum where VIII C is completely lost but VIII R AG is preserved No such effect was seen in any of our patients

The effect of plasmin on factor VIII was investigated experimentally Small amounts of plasmin suppressed VIII C without affecting VIII R AG, while larger amounts of plasmin had a more profound effect (Table 2 and Fig 1) resulting in an altered electrophoretic mobility of VIII R AG and apparently an over estimation of the amount of VIII R AG by the quantitative electroimmunoassay The electrophoretic mobility of VIII R AG in patients with a striking discrepancy between VIII R AG and VIII C was normal The findings in these patients were therefore very similar to those in the experimental model with a weak plasmin effect on normal plasma But as also VIII R AG in serum showed a normal electrophoretic mobility a low grade thrombin activity cannot be excluded

Our study showed that determination of the ratio between VIII R AG and VIII C is useful in the diagnosis of pathological proteolysis in sick newborns A low grade plasminogen activation seems the most tenable explanation for our findings Since the ratio did not differ between the patients who died and those who survived it is of no prognostic value and should not be used as the only indication for treatment with inhibitors of plasminogen activation

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ELECTROLYTES IN NAILS ANALYSED BY X RAY MICROANALYSIS IN ELECTRON MICROSCOPY

Considerations on a New Method for the Diagnosis of Cystic Fibrosis

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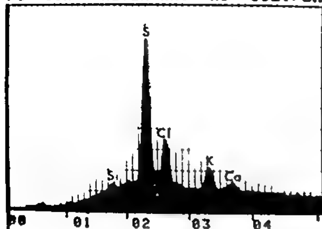
ABSTRACT Roomans G M Afzelius B A Kollberg H and Forslind B (Wenner Gren Institute University of Stockholm Sweden Department of Paediatrics University of Umeå Sweden and Department of Medical Biophysics Karolinska Institutet Stockholm Sweden) Electrolytes in nails analysed by X ray microanalysis in electron microscopy Considerations on a new method for the diagnosis of cystic fibrosis Acta Paediat Scand 67 89 1978 —Patients with cystic fibrosis (CF) have an increased concentration of sodium in their nails Hitherto only neutron activation analysis has been considered for the diagnosis of cystic fibrosis by analysis of electrolytes in nails It has been thoroughly tested methodologically and clinically However the intrinsic advantages of X ray microanalysis and the results obtained in this study suggest that this method after further testing may be a useful diagnostic aid for cystic fibrosis In comparison with neutron activation analysis X ray microanalysis has the advantage of simultaneously giving the concentrations of several elements and may be accessible at any hospital with an electron microscope fitted with the necessary equipment Nails of CF patients are here shown to have increased concentrations of Na K and Cl which will make the diagnosis of cystic fibrosis more reliable The possibility of using sulphur as a reference element may eliminate the weighing procedure necessary in neutron activation analysis

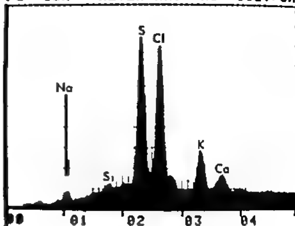
KEY WORDS Cystic fibrosis diagnostic methods nail clippings energy-dispersive X ray microanalysis sodium potassium chlorine sulphur

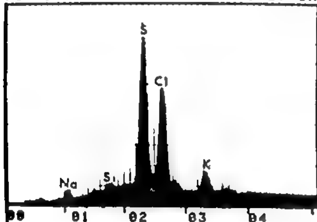
Early diagnosis and treatment of cystic fibrosis (CF) may improve the outcome for the patients (7 19) CF patients have higher concentrations of sodium in sweat (3) and nails (15 17) Neutron activation analysis of sodium in nails has proved to be a valuable diagnostic method in children over one year of age provided a proper collecting method is used (12 13 15 16) However it has the drawback that contaminations and undue soakings are difficult to reveal Furthermore it requires access to a nuclear reactor

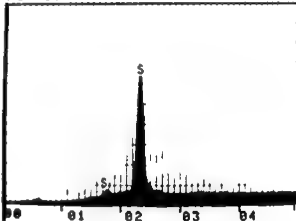
The X ray microanalysis in electron microscopy has the potential to analyse sodium as well as all elements heavier than sodium at the same time Neutral and charged species are registered simultaneously The method can be used for dried down liquid micro-samples as well as for solid structures such as tissue sections or nail clippings

The human nail is composed of two structural entities the elastic dorsal nail plate which consists of flat cells adhering closely to each other and the soft pliable ventral nail plate There are open intercellular spaces in the ventral nail plate and the available surface of this part of the nail is large in comparison to that of the dorsal nail plate (5)









Figs 1–4 X ray energy spectrum of the dorsal nail surface The most prominent peaks have been labelled with their elemental symbol *Fig 1* Spectrum from the nail of a healthy child No or little sodium has been recorded *Figs 2 and 3* Spectra from two cases of cystic fibrosis Clear-cut sodium peaks are seen Note that the detector efficiency is low below element 15 e g Si compared with Cl and K *Fig 4* Spectrum from a washed nail from a cystic fibrosis patient It is evident that elements Na Cl and K have been removed

The most reliable diagnostic test for cystic fibrosis is still that of sweat electrolyte concentrations according to Gibson & Cooke (8) However this test is difficult to perform and necessitates special equipment and trained personnel A diagnostic test for cystic fibrosis using nail clippings is therefore highly desirable This could give every child with suspected symptoms a chance to have the diagnosis tested, even those for whom a sweat test is difficult to perform e g for reasons of distance to a suitable hospital with facilities for the test

The aim of the present investigation has been to explore the possible use of electron microscopical X ray microanalysis of nails in the diagnosis of cystic fibrosis

MATERIAL AND METHODS

The free edges of finger nails were clipped from 6 cystic fibrosis patients and 4 controls all of whom were more than 3 years old (In the following nails or nail clippings always mean the free edges of the nails) The collection routine was well standardized no swimming was allowed for a week no bath in the 24 hours before clipping The nails were soaked or rinsed in the evening before clipping and mechanically cleaned immediately before clipping in the morning

The diagnosis of cystic fibrosis had been established unequivocally by double determinations of sweat electrolytes by pilocarpine iontophoresis (1 8) in addition to the well known clinical manifestations The results of the clinical diagnosis were not revealed to the non-clinical

scientists until the raw data of X ray microanalysis were tabulated

The nail clippings with known orientation were mounted on carbon plates by means of a graphite solu tion (Aquadag/KEVEX) The specimens were analysed without further treatment Data were collected from the dorsal and ventral exposed surfaces of the nail

En rgy-dispersive X ray microanalysis was performed with a KEVEX spectrometer in combination with a JEOL 100C electron microscope provided with a JEOL ASID scanning attachment Under standard operating condi tions the nail clippings were examined in the scanning mode at 40 kV (11) at a magnification of 300 times The take-off angle was 30–40° The analysis was carried out by scanning the beam over a square surface with 50 μm sides The total counting time was ?00 seconds (live) at a count rate of ?00–500 counts/second The relative peak intensity (*R*) for an element was calculated from $R=(P-b)/b$ where $(P-b)$ is the number of specific counts and b is the background under the peak (9)

The relative efficiency (sensitivity) of the detector system for the various elements was determined by the following method Small droplets of a mineral salt solu tion containing at least two of the elements Na Al P S Cl K or Ca in known atomic ratios were dried on carbon plates and analysed under the same conditions as the nails *From the intensity ratios of the elements the* relative efficiency (taking Ca=100) versus the atomic number could be plotted (2 18)

The relative efficiency of the detector system has been measured as a function of atomic number under the ex perimental conditions applied (cf Fig 5)

RESULTS

Data obtained from the dorsal side of the nails and corrected for the detector efficiency are given in Table 1 The same data correlated to sulphur as a reference element are given in Table 2

Table 1 *Measured relative intensities from the dorsal side of the nails corrected for the detector efficiency*

Figures within parentheses refer to the number of measurements

	Cystic fibrosis patients (__)	*Control group (11)*
Na	1 60±0 16	not det
P	0 08±0 03	0 08±0 0?
S	3 83±0.–3	3 69±0 36
Cl	? ?8±0 13	0 41±0 09
K	1 –8±0 14	0 40±0 09
Ca	0 48_0 06	0 64±0 07

Table 2 *Same data as in Table 1 correlated to sulphur as a reference element*

	Cystic fibrosis patients (?2)	Control group (11)
Na	4?±4	not det
P	2±0 7	2± 0 5
S	100±6	100±10
Cl	?9±3	11± 2
K	33±4	11± ?
Ca	12±2	17± 2

Nails from cystic fibrosis patients had significantly elevated concentration of Na ($p<0\ 01$) Cl ($p<0\ 01$) and K ($p<0\ 01$) (Figs 2 3 and 4) On the other hand S P and Ca were found in concentrations corresponding *to those of the controls*

The effect of rinsing on cystic fibrosis nails in distilled water is shown in Table 3 and Fig 4 The low concentrations of Na Cl K and Ca show these elements to have been washed out whereas the S concentration has been constant A control (normal nail) gave the same result on rinsing

The elements from the ventral surface of the nails were also analysed and had about the same concentrations (Tables 4 and 5)

DISCUSSION

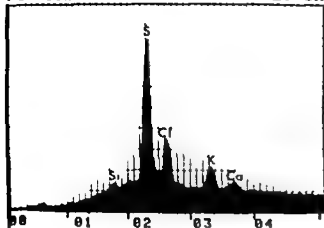
Technical considerations

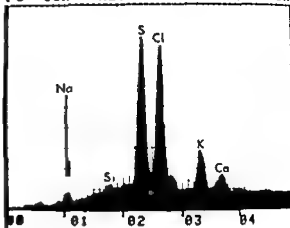
In nails sodium has been in focus for the diag nosis of cystic fibrosis If further elements

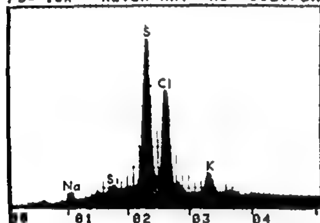
Table 3 *The effect of rinsing on nails from cystic fibrosis patients*

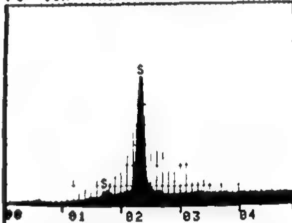
Figures within parentheses give the number of measured nails The values have been correlated to sulphur as the reference element

	Before rinsing (7)	After rinsing (4)
Na	56±7	3±2
[redacted]	2±1	2±1
S	100±5	100±6
Cl	69±3	3±1
K	43±4	2±0 5
Ca	15±3	±1









Figs 1–4 X ray energy spectrum of the dorsal nail surface The most prominent peaks have been labelled with their elemental symbol *Fig 1* Spectrum from the nail of a healthy child No or little sodium has been recorded *Figs 2 and 3* Spectra from two cases of cystic fibrosis Clear-cut sodium peaks are seen Note that the detector efficiency is low below element 15 e g Si compared with Cl and K *Fig 4* Spectrum from a washed nail from a cystic fibrosis patient It is evident that elements Na Cl and K have been removed

The most reliable diagnostic test for cystic fibrosis is still that of sweat electrolyte concentrations according to Gibson & Cooke (8) However, this test is difficult to perform and necessitates special equipment and trained personnel A diagnostic test for cystic fibrosis using nail clippings is therefore highly desirable This could give every child with suspected symptoms a chance to have the diagnosis tested, even those for whom a sweat test is difficult to perform, e g for reasons of distance to a suitable hospital with facilities for the test

The aim of the present investigation has been to explore the possible use of electron microscopical X ray microanalysis of nails in the diagnosis of cystic fibrosis

MATERIAL AND METHODS

The free edges of finger nails were clipped from 6 cystic fibrosis patients and 4 controls all of whom were more than 3 years old (In the following nails or nail clippings always mean the free edges of the nails) The collection routine was well standardized no swimming was allowed for a week no bath in the 24 hours before clipping The nails were soaked or rinsed in the evening before clipping and mechanically cleaned immediately before clipping in the morning

The diagnosis of cystic fibrosis had been established unequivocally by double determinations of sweat electrolytes by pilocarpine iontophoresis (1 8) in addition to the well known clinical manifestations The results of the clinical diagnosis were not revealed to the non-clinical

Table 4 *Measured relative intensities from the ventral side of the nails corrected for the detector efficiency*

Figures within parentheses refer to the number of measurements

	Cystic fibrosis patients (11)	Control group (2)
Na	1 50±0 30	not det
P	0 34±0 06	0 51
S	3 29±0 14	3 69
Cl	2 39±0 77	0 55
K	1 05±0 15	0 74
Ca	1 04±0 17	0 88

K intracellularly The most likely sources therefore seem to be the extrinsic and the contaminative sources This also agrees with the conclusion by Kollberg & Landstrom (16)

A hypothesis that the nail may function as an ion exchange matrix with respect to cations has been proposed by Forslind et al (6) In the present study we have plotted the regression line of the sum of the corrected intensities of Na and K to that of Cl and found that there is a linear relationship (Fig 6) The fact that the line passes through the origin indicates that Na and K^+ are laid down as NaCl and KCl From the plot it is probable that Ca^{++} is adsorbed to the nail matrix having no accompanying anion as all the Cl is taken care of by the K and Na

The ion exchange hypothesis also explains the inhomogeneity of concentrations recorded here and by several authors (4 10 14) One

Table 5 *Same values as in Table 4 although correlated to sulfur as the reference element*

Figures within parentheses refer to the number of measurements

	Cystic fibrosis patients (11)	Control group (1?)
Na	46±9	not det
P	10±	14
S	100±4	100
Cl	[illegible]±8	15
K	3 ±5	0
Ca	31±5	4

may also expect different behaviour from mono- and di valent cations in relation to this organic ion exchanger analogous to what is found in man made ion exchange matrices We find that the amount of calcium in the dorsal nail plate is lower than that of the ventral plate the latter having a potentially greater ion exchange surface (compare Tables 2 and 4)

During this study we preferred the dorsal side or the freshly cut surface for analysis because of a more favourable geometry The rough ventral surface may cause self absorption of the X rays emerging from the tilted specimen

The values of the controls compare reasonably well with those published before (6) the mineral content is somewhat less which might be explained by the degree of washing (16 18)

We have in accordance with previous investigations (17) found elevated concentrations of Na Cl and K in the nails from cystic fibrosis patients This agrees with the finding that these elements have increased concentrations in sweat from these patients and is compatible with the idea that they are of extrinsic origin The notable differences in concentrations suggest that results by X ray microanalysis in electron microscopy of elevated Na Cl and K in nails are diagnostic for cystic fibrosis However definitive border lines and estimations of diagnostic efficiency cannot be set until extensive series have been performed The concentration of calcium might be a good indicator for undue soaking (=too low values) and for possible contaminations (=too high values) More extensive methodological studies must thus be undertaken to ascertain the diagnostic potential of X ray microanalysis in the electron microscope

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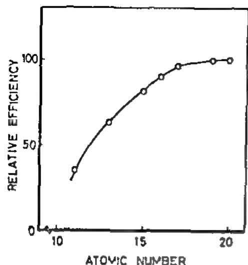


Fig 5 Plot of the relative efficiency of the detector to atomic number in the actual experiments (40 kV acceleration voltage)

could be used a higher degree of accuracy would be expected With the neutron activation analysis only sodium is analysed whereas the X ray microanalysis method provides data on a great number of elements simultaneously If the detector is calibrated with standards it is feasible to present the data in quantitative form It is to be noted that the sensitivity of the detector for Na is only a third of that of the more heavy elements K and Ca (Fig 5) This means that any significant differences between the examined nails and the normal nails for these heavier elements might be easier to detect than for sodium Such elements may prove to be as important as the sodium values for an accurate diagnosis

Analysing nails for the diagnosis of cystic fibrosis is difficult with neutron activation analysis due to the lack of long lived reference elements It is therefore necessary to determine the sodium amount in relation to the weight of the nail clipping This weight is dependent on the hydration of the nail Such factors do not enter the calculation of X ray microanalysis data where all elements present in sufficient amounts are recorded simultaneously Sulphur seems to be a good reference element In neutron activation analysis the entire cross section of the nail and the entire nail clipping is analysed In X ray microanalysis the electron beam hits a rather small area of the specimen and penetrates to a depth of about 10 μm Most X rays to be analysed are derived from a depth of about 5 μm

Bio medical considerations

The origin of Na K Ca and Cl in nails may be from three sources the intrinsic part built into the nail plate either from the matrix or from the hyponychium the extrinsic part derived from other autobiological sources such as sweat and the contaminative part from environmental sources such as table salt soap sea water salt solution etc

From a physiological point of view it seems highly unlikely that a higher than normal content of Na occurs in the living cells of growing nails The balance of the intra and extra cellular electrolytes is the pivoting axis of homeostasis Due to the osmotic effects we can hardly expect a normal protein synthesis in man in an environment with increased Na or

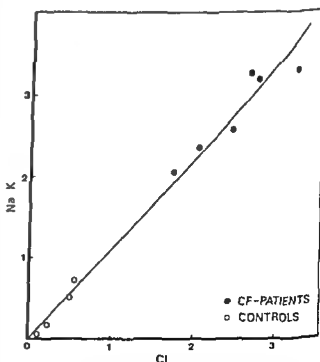


Fig 6 Corrected intensities for Na and K versus the corrected intensity of Cl in 10 examined nails The regression line passes through the origin indicating that the elements occur as NaCl and KCl in the dry nails

Table 4 *Measured relative intensities from the ventral side of the nails corrected for the detector efficiency*

Figures within parentheses refer to the number of measurements

Cystic fibrosis patients (11)		Control group (2)
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Table 5 *Same values as in Table 4 although correlated to sulfur as the reference element*

Figures within parentheses refer to the number of measurements

Cystic fibrosis patients (11)		Control group (1)
Na	46±9	not det
P	10±7	14
S	100±4	100
Cl	77±8	15
K	3±5	70
Ca	31±5	4

ACKNOWLEDGMENT

This work was supported by grants (to B A A) from the Wallenberg Foundation and the Swedish Natural Science Research Council and (to B F) from Karolinska Institutets Fonder

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THE INFLUENCE OF AMPHETAMINE ADDICTION ON PREGNANCY AND THE NEWBORN INFANT[1]

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From the Department of Pædiatrics Karolinska Institutet St Goran s Hospital Stockholm Sweden

ABSTRACT Eriksson M Larsson G Winbladh B and Zetterstrom R (Department of Pediatrics Karolinska Institute St Goran s Hospital Stockholm Sweden) The influence of amphetamine addiction on pregnancy and the newborn infant Acta Paediatr Scand 67 95 1978 —The influence of amphetamine addiction on pregnancy and the newborn infant has been studied retrospectively in 23 cases Six of the mothers claimed to have discontinued their abuse in early pregnancy while the remaining 17 mothers continued throughout In comparison with the average number of visits by pregnant Swedish women to maternal health centres the 17 women who continued their abuse made significantly fewer visits although there was a wide variation Complications related to pregnancy and delivery were few however One child with a myelomeningocele was stillborn Six children were preterm and three were small for gestational age Two full term children were extremely drowsy and in need of tube feeding symptoms that might be due to the maternal abuse Eight of the ten mothers who had previous children placed in foster homes by the Social Welfare Department left the hospital with their newborn infant in their care as well as all the eleven primiparae

KEY WORDS Maternal abuse amphetamine addiction pregnancy newborn infants custody

During the last two decades there has been increasing concern about drug dependency during pregnancy due to the effect on the fetus and the psycho-social situation for the baby The syndrome exhibited by newborn infants to mothers addicted to heroin is well documented in many publications (1 11 16) In contrast the consequences of amphetamine abuse during pregnancy have not received much interest Only a few reports on isolated cases have appeared (5 8–10 14)

Drug abuse—mainly intravenous amphetamine—increased rapidly in Sweden during the years 1960–1970 The situation is now fairly stable with a total number of about 10 000 addicts About one third of these addicts are fertile women and most of them live in the metropolitan area of Stockholm Using these figures as a background it would appear reasonable to assume that a significant number of children are born to mothers addicted to amphetamine Some social and medical aspects on amphetamine abuse during pregnancy are presented in this retrospective study

MATERIALS AND METHODS

Information has been collected retrospectively on 23 infants born in the Stockholm area to mothers addicted to amphetamine and with a diagnosis of narcomania matris in the maternity records The infants were classified in two groups according to the intensity of the maternal drug abuse during pregnancy *Group A* consists of infants born to 17 mothers who continued their amphetamine abuse throughout pregnancy *Group B* consists of infants born to 6 mothers who claimed to have discontinued their

[1] Supported by a grant from the Solstickan Foundation Stockholm

Table 1 *Previous pregnancies*

Abortions		No of deliveries	
Spontaneous	Induced	1	2–4
Group A (17 subjects)			
2	5	6	4
Group B (6 subjects)			
2	3	2	–

abuse after becoming aware of pregnancy or from the second trimester

Information was collected about maternal health pregnancy delivery and the newborn infant from the records of the maternal health centre maternity and pediatric clinics We made special note of the custody of previous children and of the present infant in order to provide background material for an opinion on the social situation

RESULTS

The age distribution of the mothers ranged from 18 to 39 years (mean 24) in Group A 22–32 years (mean 25) in Group B In more than half of the mothers (13/23) there were case histories of infectious hepatitis and gonorrhoea (10/23) Apart from this we found no indications of serious diseases in the records

Table 1 presents data on earlier pregnancies and deliveries Most of the mothers had been pregnant earlier a considerable proportion (12/31) of the pregnancies were terminated by abortion Most of them were induced (8/12) In the two groups 12 mothers had delivered a total of 19 children

Information on the prenatal care is presented in Table 2 The number of visits to the prenatal clinic varies greatly More than half of the mothers in Group A (10/17) had less than four health controls 2 of these had none In comparison with the average number of visits by pregnant Swedish women to maternal health centres the women in Group A made significantly fewer visits (5 versus 11) In contrast all 6 mothers in Group B visited the maternal health centre more than four times and the first health control was made an average of 2 months earlier than for group A (13th versus 21st week respectively) Toxaemia of pregnancy with oedema and proteinuria but not associated with elevated blood pressure was found in 3 cases all in group A

There were six preterm births with a gestational age of less than 37 weeks (all in group A) There were two breech deliveries one emergency Caesarean section due to signs of foetal asphyxia and 2 cases of postpartum haemorrhage due to retention of placenta One child with myelomeningocele was stillborn Apgar scoring at one minute was above seven in all but one child As seen in Fig 1 the weights and lengths at birth were generally appropriate for the gestational age The 3 babies below two standard deviations in weight were born to mothers who visited the maternal health centre less than four times all in group A

Half of the children in Group A (8/16) were

Table 2 *Prenatal care*

Antenatal clinic		Drug clinic contact		
<4 visits	First visit—week of pregnancy median and range	In patient	Out patient	Complications
Group A (17 subjects)				
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Group B (6 subjects)				
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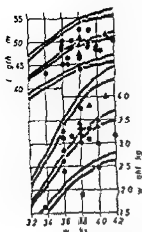


Fig 1 Length and weight at birth of ?? children of addicted mothers The corresponding parameters for ish children are indicated Mean values ±2 standard tions are given —— Boys girls (Engstrom L jerg B Olsson T Selstam U 1971 copyright ission granted by P Karlberg Department of iatrics Östra sjukhuset Gothenburg Sweden) ● :en children of group A (amphetamine abuse during whole pregnancy) ▲ Six children of group B)hetamine abuse only during the first trimester)

nsferred to paediatric wards because of pre turity low birth weight or for observation ile a psycho-social investigation of the ither s ability to care for the baby was made ily one infant in Group B was transferred d this was because of hyperbilirubinaemia ie infant in Group A with a gestational age 40 weeks and a birth weight of 2 500 g had neralized seizures of unknown etiology sting 15 min on the first postnatal day Two ill term infants belonging to Group A were noted to be drowsy and needed tube feeding for one to three days In addition they were moderately tachypnoeic Four infants had hyperbilirubinaemia (>270 µmol/l) and 2 of them were subjected to exchange transfusion

The decisions made by the Social Welfare Department concerning the mother s or parent s ability to care for the present child are shown in Table 3 Of the 12 mothers with previous children who now left the hospital with their baby only 2 had their previous children in their custody In Group A 4 infants were after care at the hospital placed in an institution while awaiting a suitable foster home In Group B on the other hand all children left the maternity wards with their mothers

DISCUSSION

Since the present study is confined to a selected group of patients where the addiction was known and recorded it cannot be used to estimate the incidence of amphetamine addiction during pregnancy However judging by the severity of the abuse in the group that has been studied it is not likely that the average complication frequency for amphetamine abuse in pregnancy delivery and the newborn is underestimated

An increased incidence of toxaemia of pregnancy and obstetrical complications have been reported in heroin addicted mothers (13) The complication rate seems to be lower in those

'able 3 *Custody and residence at discharge*

'revious children umber of mothers/ umber of children		Present children		Residence after discharge		
Foster home	Biological mother s custody	Foster home	Biological mother s custody	Mother s dwelling	Grand parents home	Institution
G oup A (16 subjects)						
8/14	?/3	4	1?	7	4	1
G up B (6 subjects)						
-/?	–	–	6	5	1	–

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Abortions		No of deliveries	
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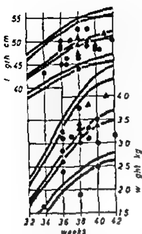


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transferred to paediatric wards because of pre maturity low birth weight or for observation while a psycho-social investigation of the mother s ability to care for the baby was made Only one infant in Group B was transferred and this was because of hyperbilirubinaemia One infant in Group A with a gestational age of 40 weeks and a birth weight of 2 500 g had generalized seizures of unknown etiology lasting 15 min on the first postnatal day Two full term infants belonging to Group A were noted to be drowsy and needed tube feeding for one to three days In addition they were moderately tachypnoeic Four infants had hyperbilirubinaemia (>270 μmol/l) and 2 of them were subjected to exchange transfusion

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who are enrolled in methadone treatment programs perhaps due to better prenatal care (2) In the present study all but two mothers visited the maternity health centre although more than half of them paid less than four visits However the complication rate for the amphetamine addicted mothers was low and seems to be lower than the rate reported previously for heroin addicted mothers (13)

The risk for teratogenicity caused by addicted drugs has been discussed especially for the amphetamines There are a few reports on different kinds of congenital malformations in babies to mothers taking amphetamine mainly as an appetite suppressor (5 9) One stillborn infant in our study had a myelomeningocele and another child had extensive telangiectasis The material is however too limited to permit any conclusions as to whether amphetamine use correlates to an increased incidence of malformations

Low birth weight is a frequent finding in infants born to mothers addicted to heroin (4 6) Three out of 22 babies in our study were small for their gestational age They were born to mothers who abused amphetamines throughout their pregnancy and visited the prenatal clinic less than four times A decreased incidence of jaundice has been reported in infants born to mothers addicted to heroin This was not found in the babies of the mothers enrolled in methadone treatment programs (7 16) Four of 22 infants in the present study were markedly jaundiced (>270 μmol/l) Two of them had to undergo exchange transfusion the others were treated with phototherapy

The withdrawal syndrome is well recognized in infants newly born to mothers abusing heroin (1) Since withdrawal symptoms differ following heroin and amphetamine abuse in adults it would seem reasonable to expect the same difference in the newborn infants (3) Discontinuation of amphetamine abuse causes dysphoria and a significant lassitude and corresponding withdrawal symptoms should be looked for in the newborn In the few case reports that have been published both agitation and drowsiness were found (8 10 14) Two of the full term children in the present study showed the latter symptom with exceptional lassitude necessitating tube feeding In addition it cannot be ruled out that the seizures observed in one infant were part of a withdrawal syndrome

The 12 mothers who had previous children continued their pregnancy in spite of the fact that only two of them raised their previous children It has been noted earlier that female addicts frequently exhibit a marked desire to have a baby (15) Sardeman et al (12) have also shown that in a study 7/19 addicted mothers felt a responsibility towards their children that helped them to discontinue their drug addiction and that the fear of having their child taken from them had prevented them from further abuse In the present study only 6/23 mothers claimed that they gave up their abuse of amphetamines in connection with pregnancy This statement must of course be evaluated cautiously but the 6 mothers in question also differed from the remaining 17 in Group A in other respects viz they came earlier for their first visit to the maternity health clinic and on average paid more visits Furthermore a higher proportion of them (5/6 versus 6/17) had arranged for a home of their own to return to together with the baby after discharge It would be of great interest to know if and to what extent this group relapses or whether the infant is a sufficient motivation for permanently abandoning drug abuse

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CASE REPORT

DELETION SHORT ARM 18 AND SILVER RUSSELL SYNDROME

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ABSTRACT Christensen M F and Nielsen J (Department of Paediatrics Central Hospital Herning and the Cytogenetic Laboratory Århus Psychiatric Hospital Risskov Denmark) Deletion short arm 18 and Silver Russell syndrome Acta Paediatr Scand 67 101 1978.—A 14-year-old boy presented with physical signs of the Silver Russell syndrome He further had low set large protruding ears ptosis broad nasal base thick protruding lips pronounced caries malalignment of teeth micrognathia and mental retardation Chromosome examination showed deletion of the short arms of chromosome No 18 It is concluded that considering the previous findings of chromosome No 18 aberrations in some patients with Silver Russell syndrome such patients should always have chromosome examination made

KEY WORDS Deletion short arm 18 autosomal chromosome abnormality Silver Russell syndrome hemihypertrophy

Patients with the Silver Russell syndrome usually have a normal karyotype some cases have however been described with chromosome abnormalities (2 6) including trisomy 18 mosaicism (1 3) We present a case of deletion short arm 18 which was initially diagnosed as Silver Russell syndrome

CASE REPORT

Case history

A 14 year-old boy had been born two weeks before term by breech delivery after an uncomplicated pregnancy At the time of his birth his mother s age was 38 years His weight was 1750 g and his crown–heel length 43 cm He was placed in an incubator for 10 days and stayed in hospital for 5 months because of failure to thrive At the age of 3 months he was examined because of suspicion of hydrocephalus which however was not confirmed

His developmental milestones were achieved later than normal he could not walk till the age of 2 and he was late in talking At the age of 5 an operation for ptosis was performed When 8 years old he was treated for marked caries At the age of 10 he was at 1 6 cm tall which was below the 3rd percentile in height

At 14 years of age he weighed 48 kg and was 149 cm tall (between the 3rd and 10th percentile) He had a brachycephalic head with normal occipito frontal circumference low set large somewhat protruding ears a deep-set broad nasal bridge large spaces between teeth and

Table 1 *Typical clinical findings in the Silver Russell syndrome (5)*

Clinical findings	Present case
Significant asymmetry	+
Shortness of stature	−
Small size despite birth at term	+
Variation in the pattern of sexual development	
Elevated urinary gonadotrophins	+
Early sexual development	−
Premature estrogenization of urethral or vaginal mucosa	
Retarded epiphyseal maturation	−
Café au lait areas of the skin	(+)
Unusually short fifth fingers and/or	+
Increased curve of fifth fingers	−
Triangular shape of face	+
Turned-down corners of the mouth	−
Syndactylism or other abnormalities of the toes	−

able 2 *Clinical findings in 82 cases of dele on short arm 18 as well as in the present case*

linical findings	82 cases	Present case
hort stature	5?/65	–
lental deficiency	76/76	+
lat occiput	13*	–
ound flat face	3?/36	–
lypertelorism	32/53	–
tosis	30/48	+
picanthic folds	28/43	–
trabismus	13/33	–
hort broad based nose	46/51	+
ide mouth	41/45	–
rregular dentition	17*	+
Outstanding caries	27*	+
mall chin	34/40	+
ig protruding ears	55/59	+
hort broad neck	40/44	–
Deep hair line	18*	–
road chest	11*	–
ectus excavatum	17/?6	–
Kyphoscoliosis	6	–
ncurved fifth fingers	?0*	+
Muscular hypotonia	28*	–

79 cases from the literature described in a survey by Schinzel et al (4) to which 3 of their own were added
* Not mentioned in the other cases

mon feature in the Silver Russell syndrome (Table 2)

The Silver Russell syndrome is an acknowledged clinical entity There is however no pathognomonic feature and the aetiology is unknown The patient fulfils minimal diagnostic criteria of this syndrome (5) Nevertheless he certainly also had deletion short arm 18 The presence of two rare diseases may be a coincidence but as other examples of the Silver Russell syndrome in patients with chromosome 18 abnormalities are known chromosome examination should always be carried out in cases of this syndrome

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Fig 1 Proband at the age of 14 years

irregular teeth position furthermore he had micrognathia thick protruding lips and a rather large mouth His left leg was 4 cm shorter than the right and his left arm 5 cm shorter than the right There was hypotrophic development of the left side of his body extremities and face compared with the right Both fifth fingers showed camptodactyly Moderate syndactyly between the second and third toe on both sides was noted He had small testes located in the inguinal canals and a small scrotum but more body hair growth than usual for boys of his age Scattered on his skin atypical café au lait areas were found (Fig 1)



Fig 2 Chromosomes Nos 17 and 18 from karyotyp 46 XY del(18)(p11) BUDR acridine-orange

Mentally he was somewhat immature for age but h appeared well adjusted in a class for retarded children i elementary school and in his family There were indica tions of space form perceptual deficits

Laboratory examinations showed high serum concer trations of pituitary gonadotropins (LH 111 μg/l FSH 1660 μg/l) Serum testosterone was at the lower end of the normal range and increased 6 times on stimulatio with chorionGonadotrophin Immunoglobulins were with in the normal range Skeletal maturation varied betwee 10 and 14 years The electroencephalogram showed dominant activity of 7–8 c/s scanty 8–9 c/s and som 4–6 c/s activity The conclusion was however that th EEG showed nothing definitely abnormal considering hi age

Cytogenetic examination

Chromosome examinations were made on 48 hour lym phocyte cultures stained with Quinacrine as well as wit BUDR acridine-orange Deletion short arm 18 with dele tion at p11 was found in all cells and the karyotype wa thus 46 XY del(18)(p11) as shown in Fig 2 Chromo some examination of the parents showed normal findings

DISCUSSION

This patient was diagnosed as suffering from the Silver Russell syndrome because he showed 1) small size despite birth only two weeks before term 2) body asymmetry 3) camptodactyly 4) syndactyly 5) café au lait areas and 6) elevated levels of serum gonado trophins (Table 1)

Some signs not usually seen in Silver Rus sell syndrome but often in deletion short arm 18 were however found i e 1) low set large protruding ears 2) ptosis 3) broad nasal bridge 4) thick protruding lips 5) marked caries 6) irregular dentition 7) small chin

Table 2 *Clinical findings in 82 cases of deletion short arm 18 as well as in the present case*

Clinical findings	82 cases	Present case
Short stature	57/65	−
Mental deficiency	76/76	+
Flat occiput	13[b]	−
Round flat face	35/36	−
Hypertelorism	37/53	−
Ptosis	30/48	+
Epicanthic folds	28/43	−
Strabismus	13/33	−
Short broad based nose	46/51	+
Wide mouth	41/45	−
Irregular dentition	17	+
Outstanding caries	22[b]	+
Small chin	34/40	+
Big protruding ears	55/59	+
Short broad neck	40/44	−
Deep hair line	18[b]	−
Broad chest	11[b]	−
Pectus excavatum	17/76	−
Kyphoscoliosis	6[b]	−
Incurved fifth fingers	20[b]	+
Muscular hypotonia	28	−

79 cases from the literature described in a survey by Schinzel et al (4) to which 3 of their own were added
Not mentioned in the other cases

mon feature in the Silver Russell syndrome (Table 2)

The Silver Russell syndrome is an acknowledged clinical entity There is however no pathognomonic feature and the aetiology is unknown The patient fulfils minimal diagnostic criteria of this syndrome (5) Nevertheless he certainly also had deletion short arm 18 The presence of two rare diseases may be a coincidence but as other examples of the Silver Russell syndrome in patients with chromosome 18 abnormalities are known chromosome examination should always be carried out in cases of this syndrome

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CASE REPORT

PELIOSIS HEPATIS IN A CHILD

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ABSTRACT Bank J Ibsen Lykkebo D and Hagerstrand I (Institute of Pathology Odense University Hospital Odense Denmark) Peliosis hepatis in a child Acta Paediatr Scand 67 105 1978 —A boy is described with peliosis hepatis He suffered from a Fanconi anaemia and was treated with prednisolone dianabol and methyltestosterone A review of the clinical and pathological aspects of the condition is given

KEY WORDS Peliosis hepatis Fanconi anaemia anabolic/androgen steroids

Peliosis hepatis is a non tumourous lesion of the liver characterized by diffuse round blood filled cystic foci (2) Peliosis is a Greek word meaning bluish black

Peliosis hepatis is normally diagnosed at autopsy It is usually a complication to a prolonged wasting disease e g adult patients suffering from tuberculosis or malignancies further it most strikingly occurs in patients treated with anabolic steroids (1) Two varieties have been described (8) 1) a phlebectatic type with aneurysmal dilatation of the central veins and 2) a parenchymal type with diffusely scattered lakes with or without fibrous lining and often associated with parenchymal necrosis A combination of these two types can be seen (1) A few cases of the disease have been reported in children (4 6) We report the condition in a boy with a Fanconi anaemia

CASE REPORT

A male child 5½ years old at death He was born 2 months before term and weighed 1700 grams He had syndaktylia of the toes (2nd and 3rd bilateral) and there was no osseous connection between the right thumb and wrist Duodenal atresia was diagnosed and operated upon in the neonatal period His karyotype was found to be normal His psychomotoric development was retarded At the age of one year aplastic anaemia was diagnosed and from his 3rd year he was treated with prednisolone (2 5 mg daily) and dianabol (0 5 mg daily) In the spring of 1976 his condition deteriorated with episodes of severe epistaxis and repeated infections In May 1976 the treatment was changed to methyltestosterone (10 mg×3) and hydrocortisone (40 mg×4) However no remission was achieved and a bone marrow transplant was considered but the patient died of septicaemia before this could be carried out Circulatory insufficiency with crural oedema developed a few days before death Liver tests were not performed

Autopsy revealed bacterial (*E coli*) septicaemia with foci in the lungs kidneys and thymus together with oesophagitis and laryngitis due to candida infection As expected the adrenal cortex was atrophic due to the prednisolone therapy The bone marrow was unchanged in relation to the findings 3 years earlier where a moderate general hypoplasia was present A horseshoe kidney and hydrocephalus were found in addition to the previously described malformations

The liver showed numerous diffusely dispersed blood filled cysts of a diameter of some millimeters (Fig 1) these were somewhat more numerous in the right than in the left lobe Microscopically the cysts were found randomly distributed in the hepatic lobules and most had the appearance of ectasies of the sinusoids without wall structures (Fig 2) Some cysts had reticulin fibres in the periphery but these were more often situated between the liver cell plates adjacent to the cysts than as a continuous layer around the cyst (Fig 3) A few cysts had the form of an aneurism of the central veins The cysts

ing from mammary, ovarian or uterine carcinoma. Peliosis has been reported as being reversible; the liver may be palpable and tender and the parameters are normal or elevated (1). Clinicians should be conversant with the disease as intra-abdominal haemorrhage has been described; similarly percutaneous liver biopsy may be hazardous (1).

The pathogenesis of peliosis hepatis is still unclear and the role of anabolic steroids, which also cause nodular regeneration and true tumours of the liver, is puzzling (5).

The most favoured theory of the pathogenesis of the condition is widening of the sinusoids after absorption of liver cell necroses, and many authors consider that right-sided heart failure is necessary for the development of the ectasies (9).

In our opinion peliosis hepatis is not rarer in children than adults, as it has been reported in ten children. In addition peliosis hepatis is mainly described nowadays following androgen therapy (3), a type of treatment rarely used in children.

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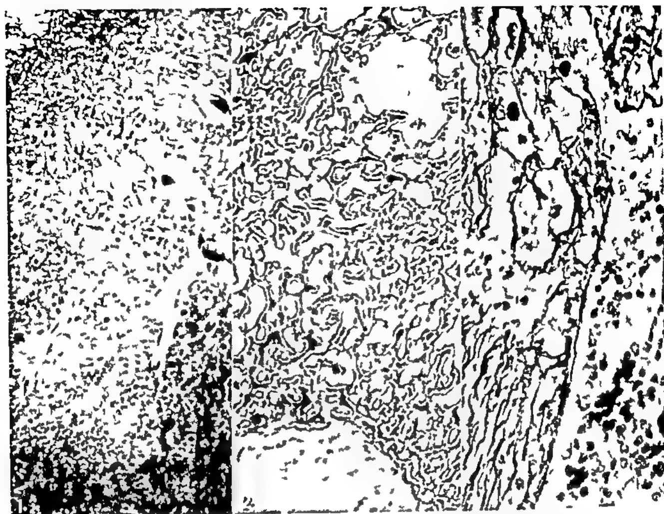


Fig 1 Macroscopic view of the liver with numerous small cysts The photograph was taken using a colour diapositive

Fig 2 Microscopic picture of the cysts showing half of a large cyst at the bottom and a smaller cyst at the right top corner There are dilated sinusoids between the cysts Reticulin stain ×30

Fig 3 A cyst continuous with hepatic sinusoids showing reticulin fibres partly in the wall and partly between adjacent hepatocytes Reticulin stain ×120

were filled with blood however no fresh or healed thrombi were observed The hepatic parenchyma was normal between the cysts except for dilatation of the centrolobular sinusoids (Fig 2) No necrosis or cholestasis were seen

The autoptical findings were consistent with the clinical diagnosis of Fanconi s anaemia (7)

DISCUSSION

Peliosis hepatis has previously been described in two children Usatin & Wigger (6) reported an 11 year old boy with cystic fibrosis of the pancreas, treated in his last 2 years with methandrosterone Nurnberger & Ramos (4) described a 15 month old female child dying of septicaemia without any other known disease and without drug intervention However the latter authors mentioned 7 other cases of peliosis hepatis in children reported from the Armed Forces Institute of Pathology

As in our patient the other two children showed a parenchymal type of peliosis hepatis but with features of the phlebectatic type also The diagnosis in all three cases was made at autopsy as is usually the case in adults The total number of cases of peliosis hepatis reported in the literature hardly exceeds 200 There seems to be no obvious sex predominance (9) even though the material of Henrikson & Odelberg (2) consisted of 23 females and 2 males Their patients had tumours treated with anabolic steroids a therapy which is used more commonly in females i e women suffer

g from mammary ovarıan or uterıne car ıoma Pelıosıs has been reported as beıng versıble the lıver may be palpable and ten r and the parameters are normal or elevated) Clınıcıans should be conversant wıth the sease as ıntra abdomınal haemorrhage has en descrıbed sımılarly percutaneous lıver opsy may be hazardous (1)

The pathogenesıs of pelıosıs hepatıs ıs stıll ıclear and the role of anabolıc sterоıds hıch also cause nodular regeneratıon and ue tumours of the lıver ıs puzzlıng (5)

The most favoured theory of the patho- nesıs of the condıtıon ıs wıdenıng of the nusoıds after absorptıon of lıver cell necro- s and many authors consıder that rıght ded heart faılure ıs necessary for the devel pment of the ectasıes (9)

In our opınıon pelıosıs hepatıs ıs not rarer ın hıldren than adults as ıt has been reported ı ten chıldren ın addıtıon pelıosıs hepatıs ıs aınly descrıbed nowadays followıng andro- en therapy (3) a type of treatment rarely sed ın chıldren

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CASE REPORT

IMMUNOSUPPRESSIVE MEASLES ENCEPHALOPATHY

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ABSTRACT Pedersen F K Schiøtz P O Valerius N H and Hertz H (University Clinic of Paediatrics Department G and TG and University Clinic of Infectious Diseases Rigshospitalet Copenhagen Denmark) Immunosuppressive measles encephalopathy Acta Paediatr Scand 67 109 1978—A case of measles infection without a rash which was followed by a severe encephalopathy after two months is described in a 2½ year old boy At the age of 8 months he had been irradiated for an inoperable intrathoracic neuroblastoma and at the time of exposure to measles he was being treated with cyclophosphamide and vincristine This case closely resembles other cases recently described and termed Immunosuppressive measles encephalopathy The syndrome is believed to represent the effect of measles virus in patients with deficient cellular immunity induced by antineoplastic treatment The importance of protecting children on immunosuppressive treatment from contracting measles is stressed

KEY WORDS Cancer therapy complications Immunosuppressive measles encephalopathy measles complications

Treatment of malignant diseases in children usually includes administration of cytotoxic drugs and radiotherapy This results in an impairment of the immunological defense mechanisms leading to an increased susceptibility to infection (4) Also the course of common infections may be atypical as well as more severe than usual (10)

This report presents a case of atypical measles infection followed by the development of a permanent encephalopathy in a boy with a neuroblastoma in remission who was being treated with antineoplastic drugs and irradiation

CASE HISTORY

The patient is a boy born in 1971 At the age of 8 months an inoperable neuroblastoma was found to be located in the left costo-vertebral angle extending into the first thoracal intervertebral space without evidence of further dissemination The tumour was irradiated with a total dose of 3300 rad out of which about 70% involved the thymus region Ten months later (July 1972) tumour cells were found in a bone marrow aspirate and therapy with vincristine and cyclophosphamide was instituted

At the age of 29 months (June 1973) the patient was exposed to measles two days after having received vincristine and cyclophosphamide He was not given human gammaglobulin and 11 days later he was admitted because of fever cough rhinitis conjunctivitis and Koplik s spots The clinical features of the disease were typical of measles including a biphasic temperature curve However a rash did not appear neither at the time of the second temperature maximum nor later On discharge he was clinically well As laboratory studies did not show evidence of tumour activity vincristine and cyclophosphamide therapy was not resumed

Eleven weeks after the admission (September 1973) the patient was readmitted because of right sided convulsions Drowsiness had been noticed for several days prior to this and during the following weeks signs of progressive cerebral disease developed with increasing lethargy universal muscular hypotonia and right sided hemiparesis A cerebral relapse of the neuroblastoma was suspected based upon the demonstration of left sided focal changes in the EEG and a slightly increased activity in the left cranial fossa media by brain scintigraphy Accordingly irradiation of the skull (total dose

3000 rad) was given and vincristine and cyclophosphamide were resumed However the patient s condition deteriorated and the treatment was discontinued after 2 months (November 1973) Further progression did not occur and subsequent studies have not revealed evidence of tumour activity The patient has remained mentally retarded with an epilepsy and a severe spastic tetraplegia although some improvement of his motor as well as intellectual function has occurred since 1974

Laboratory studies

Measles complement fixation titer in serum July 1973 0 September 1973 6 October 1973 6 June 1974 512 March 1976 1024

Spinal fluid studies September and October 1973 and March 1976 no increase in number of cells and normal concentration of protein February 1974 measles complement fixation titer 8 March 1976 measles complement fixation titer 4 concentration of albumin 0 107 g/l concentration of IgG 0 051 g/l no growth of virus by routine virus culture no evidence of oligoclonal IgG by agarose gel electrophoresis of immunoglobulin

Concentration of immunoglobulins in serum March 1976 IgG 16 4 g/l IgA 0 72 g/l IgM 0 89 g/l Concentration of albumin in serum March 1976 38 5 g/l

T lymphocytes in peripheral blood in March 1974 made up 4% in March 1976 44% of the total number of lymphocytes B lymphocytes estimated by rosette technique in March 1974 made up 21% and in March 1976 20% of the lymphocytes Estimated by immunofluorescent technique they made up 45% and 40% of the lymphocytes respectively

T lymphocytes were estimated by their spontaneous rosette formation with sheep erythrocytes B lymphocytes by their rosette formation with human erythrocytes coated with complement as well as by immunofluorescent studies for surface immunoglobulins

Vanillin mandelic acid excretion in 24 hours urine September 1971 9 2 mg (increased for age) August 1973 1 4 mg (normal for age) September 1973 1 7 mg (normal for age) March 1976 2 6 mg (normal for age)

In August 1973 bone marrow whole body X ray and whole body scintigraphy by Technetium and Gallium did not show evidence of tumour activity

In September 1973 whole body X ray and intravenous pyelography also showed no evidence of tumour activity and in March 1976 computerized axial tomography of the skull showed slightly dilated cerebral ventricles but no evidence of intracranial tumours

DISCUSSION

The usual symptoms and signs during the eruptive stage of measles and the subsequent recovery from the infection are manifestations of a complex delayed hypersensitivity reaction Humoral immunity plays a minor role in this process and a normal course of measles infection may thus be considered an index of an intact cellular immunity (2)

The suppressive effect on both cellular and humoral immunity of cancer chemotherapy alone or in combination with radiotherapy is well documented and it is more pronounced if the combined treatment is given (4) Measles in patients receiving this type of treatment may take the same course as in fully immunocompetent children However it is often atypical, prolonged severe or even fatal Thus the typical rash may be absent (8) or the disease may be complicated by giant cell pneumonia occurring as the result of a direct invasion of the lungs with measles virus (8 14) A fatal systemic dissemination of the virus with the occurrence in multiple organs of giant cells with typical inclusions has been described (11 12) *Also the antibody response* may be absent depressed or delayed and the excretion of measles virus from the upper respiratory tract may be prolonged (14)

In recent years a previously unnoticed cerebral complication to measles in the immunocompromised host has been described and the term immunosuppresive measles encephalopathy (IME) has been proposed (17) To our knowledge only 18 cases have been published (1 3 6 13 15 17 19 20) out of which 13 in children being treated for leucemia in remission 2 in children with neuroblastoma 2 in children with lymphosarcoma and one in a child with intestinal lymphangiectasis a condition also known to be associated with immune deficiency (21) Characteristically the course of the initial measles infection has been mild or atypical thus passing without a rash in 9 cases After an interval of 5 weeks to 6 months without symptoms an acute encephalopathy with lethargy focal and general convulsions hemiparesis and athetosis has developed In 16 of the 18 cases reported progression to coma and death has occurred within 1–15 weeks In the 2 surviving cases the patients were left with severe neurologic damage (13 20) Measles complement fixation titer in serum has been positive in 8 and

negative in one of 9 patients examined and cerebrospinal fluid (CSF) titer has been positive in 4 of 5 patients examined The relation of the encephalopathy to measles virus has been further substantiated by the demonstration in brain tissue of nucleocapsides of a paramyxovirus by electron microscopy (1 3 6 15 17 19 20) and of measles virus antigen by immunofluorescent studies (6 17 20)

These cases form a distinct disease entity different from what is seen in both acute measles encephalitis and subacute sclerosing panencephalitis two conditions associated with measles virus infection which also affect the CNS (16 22) Acute measles encephalitis usually occurs during the eruptive stage of the measles infection the mortality is low and severe neurologic sequelae are infrequent (23) In subacute sclerosing panencephalitis there is a mean interval of 6 years between the initial measles infection and the onset of symptoms (5) and the clinical course is slow although usually fatal

Symptoms course and laboratory findings in our case correspond to those described in IME The patient had received radiotherapy and he was being treated with immunosuppressive drugs when exposed to measles Although immunosuppression was not demonstrated in vitro during this treatment a very low percentage of T cells was found in March 1974 4 months after discontinuation of the therapy A definite exposure to measles was followed 11 days later by a febrile illness that was without a rash but otherwise typical of measles Two months later lethargy convulsions and paralysis occurred and at this time complement fixing antibodies to measles virus could be demonstrated in the serum Initially the titer was low but it rose to very high levels later a finding that has also been recorded in immunosuppressed patients with giant cell pneumonia (14)

The continued positive measles complement fixation titer in the CSF indicates the persistence of measles virus within the CNS as described also in subacute sclerosing panencephalitis (18) The ratio between the concentrations of IgG in CSF and serum related to the corresponding ratio for albumin was found increased indicating a local immunoglobulin production in the CNS (9) This is also consistent with the continued presence of a foreign antigen in the CNS The fact that measles virus could not be cultured from the CSF does not rule out its presence since virus culture from material with few cells is generally difficult

We do not see any features distinguishing our patient and the other two surviving patients with IME from the children dying from the disorder Whether the irradiation of the skull which was instituted after the beginning of the IME has changed an otherwise fatal course remains an issue of speculation

In a recent British publication the incidence of IME in children with leucemia susceptible to measles has been estimated to be 3–5% (7) Thus exposure to measles of susceptible children receiving therapy for malignant diseases should be avoided Although the administration of human gammaglobulin has proved ineffective as prophylaxis in five patients with IME (1 15 17) most authors agree that gammaglobulin should be given to these patients after a suspected exposure to measles It may be that higher doses of gammaglobulin are necessary for the prevention of measles infection in the immunosuppressed and some authors recommend two doses of 1500 mg at an interval of 48 hours to children above 3 years on immunosuppressive treatment (17)

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CASE REPORT

FARBER S DISEASE AS A CERAMIDOSIS CLINICAL RADIOLOGICAL AND BIOCHEMICAL ASPECTS

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N CREMER M TONDEUR and S PELC

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ABSTRACT Toppet M Vamos-Hurwitz E, Jonniaux G Cremer N Tondeur M and Pelc S (Departments of Paediatrics Clinical Chemistry Pathology and the Paediatric Radiology Unit Hopital Universitaire St Pierre Bruxelles Belgium) Farber s disease as a ceramidosis clinical radiological and biochemical aspects Acta Paediatr Scand 67 113 1978.—A case of Farber s disease associated with athyreosis is reported in a Belgian infant born from consanguineous parents A detailed clinical observation made from the early onset of symptoms until the death of the patient at age of 22 months together with radiological morphological and biochemical data confirmed the diagnosis of Farber s disease and its specific storage process. Cultured fibroblast studies disclosed an abnormal catabolism of ceramides presumably related to the deficiency in lysosomal ceramidase Family history confirms that the disease is inherited as an autosomal recessive trait

KEY WORDS Farber s disease X ray findings ceramides cultured fibroblasts

Farber s disease is a rare condition of child hood first observed by Farber in 1947 and re ported as a disseminated lipogranuloma tosis (10) To our knowledge only fifteen cases of this condition have been reported so far including the present patient (1 2 5 6 7 9 14 17 21 25)

Clinically it is characterized by hoarseness of the voice swollen joints with limitation of movements disseminated subcutaneous nodules and progressive cachexia Most often the symptoms are noted during the first year of life with a fatal outcome before the age of three That Farber s disease is an inherited condition is suggested by its occurrence in siblings from two families (2 10) From bio-chemical studies the most consistent finding is an intracellular storage of ceramides in sub cutaneous nodules and in several other organs (16 20 21) A deficit in acid ceramidase has been demonstrated in autopsy tissues from one case (23) and in cultured fibroblasts from three other cases (8)

The present study deals with a case pre senting characteristic signs of Farber s lipo-granulomatosis associated with athyreosis which was closely followed until death The family history of the patient further supports the hypothesis that Farber s disease is a reces sively autosomic inherited condition

This work was supported by grant no 70193 from the Belgian FRSM (Fonds de la Recherche Scientifique Médicale)

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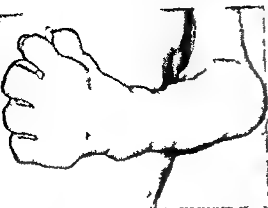


Fig 3 Patient s forearm at 1? months severe deform ity of the fingers wrists elbows



Fig 4 Elbow Muscle atrophy nodular tumefaction in the supra-condylar area joint capsular distension focal bone erosions in the olecranon process

recorded periarticular swelling of elbows knees fingers and big toes areas of osteolysis on the right olecranon (Fig 4) juxta articular bone erosions at the distal extrem ities of the ulnae and proximal extremities of the tibias irregular cone shaped deformities of the proximal ends of metacarpals and a calcified nodule on the left shoulder At 9 months a generalized micronodular infiltration of the lungs with a right apical consolidation became con spicuous (Fig 5) At the terminal stage of the disease calcified micronodules were present at the right apex the lung hili and posterior para vertebral areas

Morphological stud es

A detailed report on the pathological findings has been published elsewhere (9) Histiocytic granulomas were dis covered in the thymus lung and lymphoid tissue as well as in subcutaneous and synovial nodes There were no alterations found in the liver or kidney On electron microscopy the histiocytes appeared overloaded with irregular inclusions limited by a unit membrane and con taining mainly curvilinear structures These structures are most often composed of dense leaflets separated from each other by a clear matrix with a total thickness of 125 to 330 Å (fig 6)

Cultured fibroblasts studies

Fibroblast cultures were grown from the proband and both parents In the patient s fibroblasts morphologic studies failed to disclose any storage process both at the light and electron microscopic level When overload ing the fibroblasts with ceramides containing hydroxy fatty acids (HFA) few ultrastructural changes were in duced In contrast when the same experiment was per formed with ceramides containing non hydroxy fatty acids (NFA) typical curvilinear inclusions appeared (19) These data agree with the biochemical findings of Phil p-part et al (15) on the same cell line when labelled pre cursors of fatty acids were fed to the cells no intra cellular accumulation of HFA ceram de occurred whereas signif cant accumulation of NFA ceramides was evidenced

No accumulation was observed in normal cells or in the fibroblast cultures from the heterozygote parents A de ficient activity of lysosomial ceramidase (5% of normal values) was demonstrated by Dulaney et al on the pro band s cell line (8)

Biochemical stud es

Lipids from biopsy fragments of subcutaneous nodules were extracted as described previously (11) analysed with the solvents and specific staining recommended by Moser [redacted] (14) for ceramides and compared with standard NFA and HFA ceramides

Total lipids amounted to 4 5% fresh weight (18 5% dry weight) Thin layer chromatography revealed the pre sence of ceramides containing exclusively non hydroxy fatty acids Quantitative estimation yielded a value of *0% of total lipids* In addition cholesterol phos pholipids as well as traces of mono- and dihexoside ceramides and GM gangliosides were detected

Urinary lipid extracts submitted to TLC failed to reveal the presence of ceramides in contrast with the positive findings in the Farber case reported by Iwamori & Moser (17) A higher sensitivity of the recently developed method used by these authors might account for our failure to detect urinary ceramides

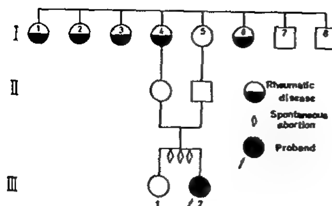


Fig 1 Family tree of the patient

CASE REPORT

The proband a Belgian female infant is the second child born to consanguineous parents first cousins The family history reveals a goitre in the mother and great aunt deaf muteness in a great uncle and an ill defined rheumatic disease in several other family members (Fig 1) The elder sister of the patient is normal

Three spontaneous abortions occurred between the births of the first and the second child During her last pregnancy the mother was treated with progestogens and was infected with viral hepatitis during the 5th month of gestation

The proband was delivered at term with a weight of 2750 g At the age of 2½ months she was admitted to the paediatric clinic with suspected hypothyroidism suggested by growth retardation puffy facies apathy and constipation A scan of the thyroid region revealed athyreosis Under thyroid hormone treatment clinical signs of hypothyroidism disappeared and statural growth resumed although it remained below the 3rd percentile Persisting hoarseness was also recorded during the endocrinological follow up

At the age of 4½ months pain on movement of the upper limbs was mentioned by the parents At 6 months a hoarse voice and a generalized muscular wasting were noted on examination At the age of 8 months the occurrence of swelling at the dorsal surfaces of the hands and feet including the first phalanges motivated the readmission to the clinic

Physical examination revealed growth retardation (weight below the third percentile 5570 g Height at the third percentile 64 5 cm) swollen metacarpo metatarso and interphalangeal joints which were painful when moved There was generalized muscular wasting and hepatosplenomegaly the border of the liver and spleen being palpable 2 cm below the costal margins Subcutaneous nodules measuring 2–3 mm in diameter were palpated on the scalp and back of the right hand There were no signs of neurological involvement and on psychomotor testing the patient s mental development seemed to be normal but there was a delayed postural control *Laboratory investigations* showed altered inflammatory tests erythrocyte sedimentation rate 48 mm/h fibrinogen 590 mg alpha 2 globulins increased to 18% of total protein and a slightly positive CRP Le cells RA test and Waaler Rose reaction were negative The 3rd complement component was markedly elevated Blood urea nitrogen uric acid and thyroid tests were normal as were urinary MPS and amino acid output No abnormal blood cells were noted in bone marrow and peripheral blood Ophthalmologic examination revealed no abnormality in the fundi lenses or cornea Direct laryngoscopy revealed thickening of the epiglottis and arythenoidal areas

While the possibility of rheumatoid arthritis might have been suggested by the combination of joint involvement and inflammatory tests the more likely diagnosis of Farber s disease was proposed by one of us (S P) in view of the patient s age peculiar joint alterations with subcutaneous nodules hoarseness of the voice and hepatosplenomegaly

With the agreement of the parents nodule skin and liver biopsies were made for ultrastructural biochemical and cultured fibroblast studies The patient was followed closely (Home Care Service) for about 14 months until her death at 22 months The terminal course of the disease was characterized by failure to thrive (weight remained at 5000 g Fig 2) Painful swelling and increasingly severe deformity of the distal joints preventing any mobilization even height measurement (Fig 3) dissemination of subcutaneous nodules the largest reaching a diameter of 4 cm a progressive muscular wasting involving predominantly the proximal groups with all deep tendon reflexes remaining normal several febrile episodes with clinical signs of pulmonary infection

In view of the failure of salicylate treatment corticosteroid therapy was attempted but with no more significant improvement At the age of 20 months respiratory distress became evident primarily due to tracheal compression by increasingly voluminous nodules Cachexy and joint swellings became increasingly prominent until death supervened

X Ray findings

Radiologic follow up of the patient began at the age of 3 months At that time the only skeletal changes were those consistent with hypothyroidism namely retarded bone maturation and heavy ring shadows on the margins of the epiphysis Beginning at 6 months of age there was a diffuse progressive bone demineralization and marked muscular wasting In addition the following changes were



Fig 2 The patient aged 12 months severe cachexy muscular wasting disseminated nodules joint swellings



Fig 6 Lung Histiocyte from a granuloma Large in clusion containing curvilinear structures (×47 000)

system nor muscles could be performed in this case the question as to whether the nervous system escaped the disease process remains unanswered

Unusual findings in our observation include the association of Farber s disease with athyreosis not hitherto reported and the strikingly altered inflammatory tests (ESR fibrinogen alpha 2 globulin) The latter data would seem to suggest a diagnosis of rheuma toid arthritis or collagenosis if it were not for the very young age of the patient Interest ing in this respect is perhaps the occurrence in many relatives of the proband of so called rheumatic disease (Fig 1) Similar findings were also reported by Moser et al (14) in the family tree of his patient

The consanguinity of the parents should be emphasized This fact strongly supports an autosomal recessive heredity of Farber s disease as already proposed by several authors (1 10) and confirmed by the recent report of Amirhakimi et al (2)

To date there have been few positive data in the family histories of the cases previously reported In one of the cases with protracted course reported by Zetterstrom (25) the defi ciency in ceramidase was also demonstrated by Dulaney (personal communication) How ever such a finding does *not necessarily* im ply for this group of patients identical muta tions such as for those with rapid evolution In this respect the situation in Farber s disease might be compared to that in muco polysaccharidoses types I and V both defi cient in alpha iduronidase (4) although their clinical pictures are quite distinct (13)

In the sibship reported by Amirhakimi et al one of the patients (Table 1 patient 10) died at the age of one whereas both others were



Fig 5 Chest Multiple nodular hilar and perihilar calcifications Right apical consolidation

DISCUSSION

In many respects the patient reported here is comparable to 9 of the 14 cases of Farber's disease reported so far (1 2 6 10 14 17 21) (Table 1)

The clinical signs are strikingly similar joint deformities multiple subcutaneous nodules hoarseness of the voice pulmonary infiltration failure to thrive leading to severe cachexia and progressive and rapidly fatal course

A slower evolution characterizes in contrast the cases reported by Zetterstrom (25) Crocket et al (7) Barrière & Gillot (5) as well as two of the siblings observed by Amirhakimi et al (2) This suggests that there may be at least two distinct modes of expression of Farber's disease

The present case represents obviously an instance of Farber's disease with rapid course Owing to the close follow up because of the coincident presence of hypothyroidism it was possible to detect at 4 months the first clinical and radiological signs of the disease and to evaluate their subsequent evolution

Clinical signs of neurologic involvement have been documented in 8 out of 9 cases with a rapid course (14) In our patient however there was no clinical evidence of either peripheral or central nervous system involvement In particular deep tendon reflexes and mental development remained normal until death Since no pathological study of the nervous

Table 1 *Clinical signs in Farber disease*

Pat no		Onset before 12 months	Hoarse voice	Joint swelling	Subcutaneous nodules	Cachexy	Pulmonary infiltration	Neurologic involvement	Death before 24 months
Rapid course (death before 24 months)									
1	Farber (10)	+	+	+	+	+	+	+	+
2	Farber (10)	+	+	+	+	+	+	+	+
3	Farber (10)	+	+	+	+	+	+	+	+
4	Abul Haj et al (1)	+	+	+	+	+		+	+
5	Schanche et al (21)	+	+	+	+	+	+	+	+
6	Rampini & Clausen (17)	+	+	+	+	+	+	+	+
7	Moser et al (14)	+	+	+	+	+	+	+	+
8	Battin et al (6)	+	+	+	+	+	+	+	+
[illegible]	Dustin et al (9)	+	+	+	+	+	+	[illegible]	+
10	Amirhakimi et al (2)	+	+	+	+	+		+	+
Delayed course									
11	Zetterstrom (25)	+	+	+	+	+	+	−	−
12	Crocker et al (7)	−	−	+	+	−		−	−
13	Barrière & Gillot (5)	+	+	+	+	+	−	+	−
14	Amirhakimi et al (2)	+	+	+	+	+		+	−
15	Amirhakimi et al (2)	+	−	+	+	−			−

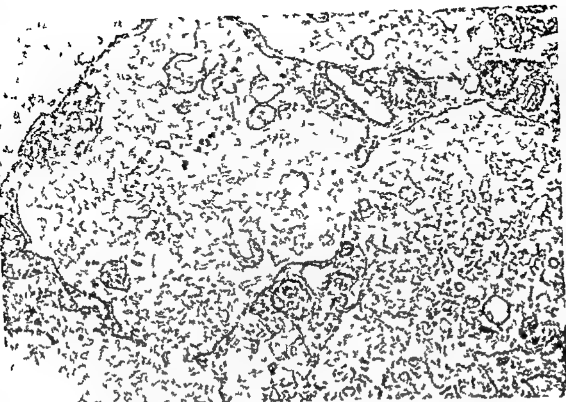


Fig 6 Lung Histiocyte from a granuloma Large in clusion containing curvilinear structures (×47 000)

system nor muscles could be performed in this case the question as to whether the nervous system escaped the disease process remains unanswered

Unusual findings in our observation include the association of Farber s disease with athyreosis not hitherto reported and the strikingly altered inflammatory tests (ESR fibrinogen alpha 2 globulin) The latter data would seem to suggest a diagnosis of rheuma toid arthritis or collagenosis if it were not for the very young age of the patient Interest ing in this respect is perhaps the occurrence in many relatives of the proband of so called rheumatic disease (Fig 1) Similar findings were also reported by *Moser et al* (14) in the family tree of his patient

The consanguinity of the parents should be emphasized This fact strongly supports an autosomal recessive heredity of Farber s disease as already proposed by several authors (1 10) and confirmed by the recent report of Amirhakimi et al (2)

To date there have been few positive data in the family histories of the cases previously reported In one of the cases with protracted course reported by Zetterstrom (25) the defi ciency in ceramidase was also demonstrated by Dulaney (personal communication) How ever such a finding does not necessarily im ply for this group of patients identical muta tions such as for those with rapid evolution In this respect the situation in Farber s disease might be compared to that in muco polysaccharidoses types I and V both defi cient in alpha iduronidase (4) although their clinical pictures are quite distinct (13)

In the sibship reported by Amirhakimi et al one of the patients (Table 1 patient 10) died at the age of one whereas both others were

still alive (patients 14–15, Table I) Because of inadequate record data it is not possible to state whether the precocious death of patient 10 was due to the disease course itself or to environmental factors Therefore the question as to whether the cases with delayed course represent a distinct genetic entity or a benign expression of the same disease can not be answered at present

In our case skeletal and pulmonary radiological findings were also consistent with Farber s disease Similar radiological features have been described by Schanche et al (21) and also by Schultze & Lang (22) the latter concerning the case subsequently reported by Abul Haj et al (1)

As for the pathological findings the inclusions containing curvilinear structures found in histiocytes from several organs (9) are similar to those described by Van Hoof & Hers in another case of Farber s disease (24) These inclusions have been reproduced experimentally in our patient s fibroblasts by overloading with NFA ceramides (19)

Biochemical investigations reveal an accumulation of ceramides in the subcutaneous nodules comparable to that reported in previous patients (14 16 20 21) In addition a *severe deficiency in acid ceramidase (± 5%* normal values) is demonstrated by Dulaney et al in fibroblasts from the present patient as well as in the three other Farber cell lines investigated (8) It should be pointed out that both ceramide storage and ceramidase deficiency have been demonstrated in all cases of Farber s disease where these parameters have been investigated Therefore these biochemical criteria represent the best clues for confirming a diagnosis of Farber s disease suggested on clinical grounds

That ceramidase deficiency represents the basic defect in this condition is further supported by the finding in the parents fibroblasts of decreased levels of ceramidase (±50%) consistent with heterozygocity (8) These enzyme data make the prenatal diagnosis of Farber s disease presently feasible

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CASE RFPORT

BILATERAL RFNAL APLASIA WITHOUT POTTER S SYNDROMF

OLA HJALMARSON and KARL-GÖRAN SABEL

From the Department of Paediatrics University of Goteborg Goteborg Sweden

ABSTRACT Hjalmarson O and Sabel K G (Department of Paediatrics University of Goteborg Goteborg Sweden) Bilateral renal aplasia without Potter s syndrome Acta Paediatr Scand 67 121 1978.—A newborn infant with bilateral aplasia of kidneys and ureters and a rudimentary bladder is reported Other manifestations of Potter s syndrome (oligohydramnios lung hypoplasia and an abnormal face) were missing as were other congenital malformations Deviations from the full picture of Potter s syndrome seem to be rare This case however shows that bilateral renal aplasia cannot be excluded as a cause of anuria in a newborn infant even if all other manifestations of Potter s syndrome are missing

KEY WORDS Potter s syndrome renal aplasia

Bilateral renal aplasia is almost always associated by oligohydramnios lung hypoplasia and an abnormal face with low set ears senile appearance small chin and a typical fold around the medical canthus of the eyes (9 10) a constellation known as Potter s syndrome It is often accompanied with other malformations Bilateral renal aplasia without one or more of the extrarenal features of Potter s syndrome have rarely been reported Published cases have had some other malformation (1 14 15) or a monoamniotic twin with urine production (6) and a normal amount of amniotic fluid has been present (Table 1)

We want to report a newborn infant with bilateral aplasia of the upper urinary tract with neither the nonrenal manifestations of Potter's syndrome nor other major malformations

CASE REPORT

The mother was a 20 year-old primigravida with an uncomplicated pregnancy until the 33rd week of gestation when she was admitted because of vaginal bleeding The growth of the uterus had been normal during the seven visits to the prenatal care clinic An ultrasound investigation two days prior to delivery showed a normal amount of amniotic fluid Cesarian section was performed A small amount of amniotic fluid was present and the male infant was found to be covered by dark green meconium Birth weight was 1770 g length 41 cm and head circumference 30 5 cm all appropriate for gestational age (3)

The boy was well nourished and had neither apparent stigmata of Potter's syndrome nor other exterior malformations (Fig 1) During the first day of life the respiratory rate was increased and a lung film showed bilateral perihilar opacities The condition normalized completely during the following day

On the second day of life edema appeared in the legs and the back No urine had been produced in spite of intravenous administration of furosemide Blood urea nitrogen was 24 mg/100 ml on the second day and 33 mg/100 ml on the third day A catheter was inserted in the urethra but did not reach the bladder At this stage the cause of the anuria was still not clear and peritoneal dialysis was started on the third day of life The boy was alert and had a normal respiration and good color and was taking expressed breastmilk by nipple

Angiography from a catheter in the left atrium showed a normal aorta descendens but no renal arteries could be visualized and no renal parenchyma could be detected On the ninth day of life an explorative operation was performed which confirmed bilateral renal aplasia Renal transplantation was not considered possible and dialysis was terminated The infant rapidly deteriorated and died on the eleventh day

The autopsy showed total renal aplasia with total absence of renal arteries and ureters A rudimentary urinary bladder with a diameter of 5 mm was present No lumen



Table 1 *Potter s syndrome and related variants*

Author	Bilateral renal agenesis	Potter face	Lung hypoplasia	Oligo-hydramnios	Other mal formations
Potter (9 10)	+	+	+	+	Common
Fantel & Shepard (4) and Perlman & Levin (7)	[illegible]	+	+	+	0
Bain & Scott (1)	+	0	+	0	+*
Sylvester & Hughes (14)	+	0	?	[illegible]	+
Thomas & Smith (15)	+	0	0	0	+
Mauer et al (6)	+	0	0	0ʳ	0
Present case	+	0	0	0	0

Information available only in 1 of 3 cases
* Severe CNS malformations
Scaphoid skull and misshapen left ear
Lungs haemorrhagic and partially aerated Hypoplasia not mentioned
Monoamniotic twin with one functioning kidney present

fluid and show a hitherto unrecognized and un explained importance of this fluid in the de velopment of fetal lungs

In one of the non oligohydramniotic cases in Table 1 the presence of amniotic fluid could be explained by a monoamniotic twin with functioning kidneys (6) In two cases fetal swallowing and reabsorption of amniotic fluid from the intestinal tract was impossible be cause of severe CNS malformation in one (1) and oesophageal atresia without a tracheoeso phageal fistula in the other (15) The nonrenal origin of the amniotic fluid found in our case remains obscure

Fantel & Shepard pointed out from their case that oligohydramnios together with a Potter habitus does not exclude normal kid neys (4) Our case shows that bilateral renal aplasia cannot be excluded as a cause of anuria in a newborn infant even if all visible mani festations of Potter s syndrome are missing

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Fig 1 The infant at ten days of age

could be identified Both lungs appeared normal and had normal weight (45 g) The lung weight/body weight ratio was normal 0 025 (13) No additional malformations or abnormalities were found Chromosome analysis with G banding from lymphocyte and fibroblast cultures was normal (46 XY)

Placental weight was 350 g the diameter 13 cm A duplication of the chorion was found around less than half the circumference giving the appearance of a circumvallate placenta No opacities or plaques were found in the membranes The histological examination was normal

DISCUSSION

The absence of Potter s syndrome in this case obscured the diagnosis for some days A normal amount of amniotic fluid during the pregnancy was indicated by a normal fundal height and growth and by ultrasonic examination but only a small amount of amniotic fluid was present at delivery Some fluid may have been lost with the discharge observed for two days preceding delivery

Lung hypoplasia was excluded as the lung function was normal after the first days and lungs of normal weight and appearance were found post mortem The face was normal and no eye folds were present Potter recently stated, that the presence of this fold is invariable (12) and in an earlier review that its absence almost always means that functioning kidney is present (11) The same has been pointed out about the presence of amniotic fluid (11) Our case is evidently an exception to these rules

Other reported variants of Potter s syndrome are presented in Table 1 There is a good correlation between oligohydramnios face abnormalities and—with a notable exception—lung hypoplasia

Oligohydramnios has been considered to be responsible for some (1 12) or all (4 15) nonrenal features of Potter s syndrome Our case is compatible with these theories The opinion that the facial signs are due to oligohydramnios has recently been criticized by Potter (12) It is however supported by recent observations of a fully expressed Potter face and lung hypoplasia in cases with normal kidneys but oligohydramnios due to chronic leakage of amniotic fluid (2 4 5 8 15) (Table 1)

Perlman & Levin (7) reported twenty one cases of various types of congenital malformations of the urinary tract with anuria These cases all had oligohydramnios and lung hypoplasia whereas patients with partial obstruction or unilateral malformations did not Their observations strongly point out the primary role of fetal urine in production of amniotic

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BOOK REVIEWS

G F Fraser *The causes of profound deafness in childhood A study of 3 535 individuals with severe hearing loss present at birth or of childhood onset* Baillière Tindall London 1977 479 pp illus £17 00 ISBN 0-7020-0640-8

The author who is professor of human genetics at the University of Leiden has for many years taken a great interest in hereditary deafness In this field he has especially contributed to our knowledge of deafness and goiter

In the present volume he presents a very thorough study of 3 535 school children who are educated in special schools or classes in the British Isles and South Australia The study is primarily concerned with severe hearing loss and profound childhood deafness leading to serious interference with the learning of speech This condition seems to affect as many as 1 person per 1 000 population in economically advanced countries The milder forms of hearing loss in childhood which affect a much larger proportion of the population are also considered to some extent The study displays a remarkable wide range of etiological factors Hereditary causes of course dominate and special chapters are devoted to deafness with goiter deafness with abnormal EKG deafness with retinitis pigmentosa X linked deafness and auditory pigmentary syndromes Also the difficult problems of genetic counseling is dealt with in a comprehensive way In the last chapter the author foresees further definitions of genetically determined deafness by identification of the gene loci involved through linkage or biochemical data

It is a pleasure to recommend this well written book to people who in their medical work are concerned with children with hearing loss

Sten Harris

K Elliott & J Knight (eds) *Acute diarrhoea in childhood* Ciba Foundation Symposium 42 Elsevier Excerpta Medica North Holland Amsterdam 1976 375 pp illus Dfl 70 00 ISBN 90-219-4047 7

The series of Ciba Foundation publications are probably wellknown to most pediatricians today since a few in recent years have dealt with specific pediatric problems such as parent–infant interaction (Symposium 33) and a volume on breastfeeding (Symposium 45) is under way

The present volume covers many aspects of our knowledge both on aetiology and therapy of diarrhoea in childhood The interdisciplinary approach in the planning of this symposium with specialists on enterotoxinogenic diarrhoea and viral gastroenteritis in young animals turns out to be very fruitful as judged from the long informal discussions after each lecture The discussions are quite vivid and show the complexity of the very dynamic field of diarrhoea research The rapidly increasing knowledge of enterotoxinogenic enteropathies is summarized in three different lectures on the mode of action of cholera toxin at the cell membrane level and on the regulation of active ion transport in the gut Most of the observations with cholera toxin are also valid for enterotoxinogenic *E coli* in infant diarrhoea which was once called cholera infantum by pediatricians long before the discovery that bacterial toxins can cause diarrhoea without any signs of inflammation in the gut mucosa This obviously makes the term gastroenteritis for diarrhoea of unknown aetiology quite dubious today which is also reflected by the choice of title for this volume

Only one chapter in the book deals with the relative importance of new bacterial agents such as toxinogenic *E coli* and viruses such as rotavirus in epidemiological studies and it is quite obvious that more studies have to be performed However it seems as if rotavirus is a very common cause of gastroenteritis in young children in temperate climates during the winter season while enterotoxinogenic agents probably are common in most tropical areas both in the dry and rainy seasons For pediatricians bacteriologists virologists and immunologists with research interests in diarrhoeal disease this volume is a must The lectures and the discussions published clearly show where we stand today and also reflect the dynamic nature of the field The increasing knowledge of both enterotoxinogenic diarrhoeas and viral gastroenteritis in young animals such as calves and piglets has permitted the development of new vaccines The results achieved will definitely be fruitful for the development of new methods for prophylaxis and prevention of diarrhoeal disease especially in developing countries

The first sentence of this volume is cited as follows During 1975 five hundred million episodes of diarrhoea were likely to occur among babies and small children of Asia Africa and Latin America and the disease would kill between five and eighteen million of them In the discussion of the last lecture with the challenging title Taking science where the diarrhoea is new approaches to prophylaxis and therapy of diarrhoea were referred to as the hedache of the developing world Our knowledge of the prophylactic role of breastfeeding and the role of treatment with glucose/electrolyte solutions in tropical countries with poor hygiene is also handled in detail The book can be fully recommended as the most up to date volume available covering most aspects of the subject The dynamics of research in this area will probably make it somewhat old fashioned already in a few years How

ever the moderate price of this Ciba Foundation Symposium should not prevent it from being added to the library of every pediatric department and clinical microbiology laboratory

Torkel Wadstrom

D S McLaren (ed) *Nutrition in the community* 395 pp John Wiley & Sons Ltd Chichester London New York Sydney Toronto 1976 £11 50

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Undernutrition will continue to dominate the spectrum of nutritional problems Therefore many wise words said by the authors who together represent a vast knowledge about the underlying causes of undernutrition deserve further exposure Already the long but interesting foreword contains some important truths Mass distribution of food is an inadequate method of treatment Food production for local consumption has often been treated as of secondary importance No amount of biostatistics and computers can replace good and regular observations Milk given out in young child clinics has damaged an excellent tradition of breast feeding Demoralisation by handout is now a common situation

The editor announces the book as providing a complete and balanced coverage of the very broad subject Nutrition in the Community Of course this promise is not really kept although undernutrition as well as over nutrition and the ways to combat them have received attention To combine in one book the nutritional problems of poor societies with those of the affluent societies seems a bit strained Even if one would like to see demand and supply of food on a global basis it does not work out that way in practice Country experiences at the end of the volume have been included in an arbitrary manner Africa is only represented by Ethiopia and South America has been omitted entirely as has the Middle East despite that many of the authors resided in Beirut The Phillipines with a barely camouflaged song of praise to the government serve to exemplify South East Asia

On the whole the book is very readable Many chapters are good or excellent for example the one on centers for combating childhood malnutrition the role of food mixtures for this purpose the interesting synopsis of new sources of food food fortification obesity the role of government in nutrition Some parts are less well covered or could benefit from revision in the next edition for example group feeding in normal and emergency situations nutrition policy and programme planning food control the role of the United Nations With multiple authorship some repetitions are unavoidable and subjects as multifactorial causation of malnutrition (chapter 7) and the epidemiology of malnutrition (chapter 8) are bound to overlap which in effect they also do

I found the book enjoyable and full of valuable infor tion and thoughts It can be recommended to paedia cians with interest in community nutrition

Gunnar Meeuwiss

Z Laron (ed) The adipose child vol 1 277 pp i *In* Z Laron & Z Dickerman (eds) *Pediatric and ado cent endocrinology* S Karger Basel Munchen P London New York Sydney 1976 sFr/DM 89 – I 3 8055 2343 2

The sessions of the 1st International Symposium o Adipose Child Israel April 1975 have appeared as th volume of a new series Pediatric and Adolescent E crinology Series and volume editor is the sympo president Zvi Laron

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The great methodological problems in measuring f size and number are presented The present methods not recognize fat cells before they have begun to acc late fat i e the role of preadipocytes in human ad tissue still remains unanswered Therefore it is very cult to evaluate the reports of trigger periods f cell multiplication in childhood obesity However data regarding the normal development of fat cells well established The normal expansion of the fat c during the first year of life is explained by an inc in fat cell size with no change in cell number

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Anders Ha

F H Stone Psychiatry and the paediatrician *In* J Apley (ed) *Postgraduate paediatrics series* Butterworths London Boston 1976 175 pp £6 00 ISBN 0407000747

Incompetence in the psycho-social sphere causes the syndrome of the frustrated paediatrician Only a better knowledge of the social and psychological aspects of family and child development can cure him Dr Stone s new book is rightly praised in the foreword by professor J Richmond Harvard Medical School It is temptingly readable experience and wisdom admirably simplified in lucid medical English

The first chapter gives the fundaments of the child s emotional development This starts immediately after birth (or before?) and is intimately bound to the mother s emotional health and attachment behaviour It can easily be disturbed by strong anxiety during labour and by separation between mother and child The infant s need of protection stimulation and consolation must be satisfied to give a basis for normal emotional and social behaviour

The chapters on neurosis personality disorders problems associated with organic brain dysfunction and child psychosis are short but eminently stuffed with facts and clinical examples Common clinical problems are exemplified and often explained

The author shows that diagnosis should not be based on exclusion alone—psycho-social and constitutional factors should be disclosed The book is practically free from psychiatric jargon or special psychiatric nomenclature unknown to paediatricians

Examples are given on an excellent interview technique starting with relaxing general questions and continuing with active listening

The author favours rooming in at hospitalisation for parents and small children He points out that the benefits are diminished or even lost if (*a*) there is a disturbed relationship between parent and child (*b*) the mother has strong reservations about living in and (*c*) if the medical and especially the nursing staffs are unenthusiastic

It should be added that all three of these call for active intervention

Modern paediatric diagnostic and therapeutical work is often based on the collaboration between paediatrician and social worker and/or a psychologist This collaboration is however hardly mentioned A more elaborate presentation of psychotherapy should also have been appropriate The reservations to this excellent book are however few and it is recommended to paediatricians in training—and in this field many of us are

Rutger Lagercrant

ANNOUNCEMENT

The IV International Symposium on Paediatric and Adolescent Gynaecology will be held in Florence Italy October 5-7 1978 For further information write to Professor V Bruni Clinica Ostetrica e Ginecologica Universita degli Studi Viale Morgagni 85 50134 Firenze Italy

ever the moderate price of this Ciba Foundation Symposium should not prevent it from being added to the library of every pediatric department and clinical microbiology laboratory

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Anders Ha...

Acta Pædiatr Scand 67 129–137 1978

SUDDEN INFANT DEATH IN COPENHAGEN 1956–1971

I *Infant Feeding*

FIN BIERING SØRENSEN TORBEN JØRGENSEN
and JØRGEN HILDEN

From the Municipal Agency of Infant Health Visitors Copenhagen and the Institute of Human Genetics University of Copenhagen Copenhagen Denmark

ABSTRACT Biering Sørensen F Jørgensen T and Hilden J (Municipal Agency of Infant Health Visitors Copenhagen and Institute of Human Genetics University of Copenhagen Copenhagen Denmark) Sudden infant death in Copenhagen 1956–1971 I Infant feeding Acta Paediatr Scand 67 129 1978 —131 cases of the Sudden Infant Death Syndrome (SIDS) in the municipality of Copenhagen 1956–71 (incidence 0 92 SIDS cases per 1 000 live births) were investigated on the basis of police reports and infant health visitor s records Fewer SIDS cases were breast fed than controls from the second week of life to four months of age No significant differences were found with respect to the concentrations of fresh cow s milk dilutions age at introduction of solid food or number of meals per day From 1956 to 1971 breast feeding was declining and solids introduced earlier while the incidence of SIDS remained constant The lifespan for SIDS cases who had never been breast fed was equal to that of cases who had There is no evidence that SIDS victims had their first solid food during the last days of life The results are discussed in the light of the hypersensitivity immuno-incompetence and high solute feeding hypotheses It is concluded that feeding does not seem to be responsible for the occurrence of SIDS The lower frequency of breast feeding among SIDS cases is ascribed to various factors known to be associated with SIDS

KEY WORDS Death sudden infant mortality infant nutrition infant food breast feeding food hypersensitivity

This paper forms part of a larger epidemiological study of the Sudden Infant Death Syndrome (SIDS) in the municipality of Copenhagen from 1956 to 1971 based mainly on prospectively collected data obtained by infant health visitors

The role of infant feeding in relation to SIDS has been widely discussed during the past few years Many epidemiological studies (2 7 16 19 27 31 34) have dealt with breast versus artificial feeding and the majority have shown a higher frequency of artificial feeding among SIDS cases than among controls

Various explanations have been suggested in particular hypersensitivity with anaphylactic reaction to cow s milk has been discussed for many years (8 9 19 27 28 29 37 39) Recently a hypothesis of high solute feeding and its relation to SIDS has received attention (14 26 30)

By using the infant health visitors carefully recorded information we are able to present more detailed data than in previous reports on the feeding pattern of SIDS cases from birth to death

On the basis of these results the named hypotheses will be discussed below

MATERIAL AND METHOD

For the delimitation of the material we adopted the following modification of the SIDS *definition* proposed by Beckwith at the Second International Conference on the

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P O Öckerman
Gorel Östberg

…months		Four months	
…s	Controls	Cases	Controls
…(1)	17 (84)	1 (1)	7 (34)
…5)	.1 (101)	3 (?)	18 (86)
…7)	6? (304)	95 (60)	75 (369)
18 21 ( ) <0 0005		11 49 (1) 0 0007	
…4)	(35)	(68)	(35)

…vere in touch with over 99% of all infants living at home …nfants placed in a crèche orphanage or similar institu…ion are not seen by the infant health visitors

For 131 accepted SIDS *cases* 44 females and 87 males …it was possible to trace the infant health visitors case re…cords These cases make up the final material while all 139 were used in calculating the incidence

Each SIDS case was matched by sex and birth date with four *controls* selected at random from the infant health visitors files The controls for 1956 are from 1957 but born on the same date and month one male case from 1958 has four female controls

The infant health visitors records contain data on infant feeding obtained prospectively at the second week of life

…ree months		Four months	
…ses	Controls	Cases	Controls
… (1)	23 (?8)	6 (1)	13 (16)
11 (?)	23 (8)	–	?1 (?6)
33 (15)	54 (66)	94 (15)	66 (80)
–	18 (19)	–	5 (6)
? (1)	[illegible] (3?)	6 (1)	22 (23)
95 (18)	5? (56)	94 (17)	73 (77)
11 (3)	[illegible] (?9)	–	6 (10)
11 (3)	17 (?7)	5 (1)	17 (6)
79 (2..)	64 (99)	95 (?1)	77 (119)
–	8 (8)	–	2 (?)
–	13 (14)	–	10 (11)
100 (1?)	79 (83)	100 (7)	88 (93)

at one month (½–1½) two (1½–2½) three (2½–3½) four (3½–4½) and six (5–7) months of age The data for the second week of life are usually derived from the infant health visitor's first visit to the home For the remaining period she makes an effort to pay her visits as near as possible to the whole months after the infant s birth But as indicated we have accepted variations up to 15 days on either side of the ideal date and one month for the 6-month visit

As to the police reports information was extracted concerning the time of the last meal the last time seen alive and the nature of the last meal

In the statistical analysis (chi square Mann Whitney rank sum tests) a five per cent level of significance was used The matching of cases and controls has not been exploited Note that statistics pertaining to different ages are *dependent in that the same life histories are being summarized*

RESULTS

Incidence

The incidence is 0 92 cases per 1 000 live births (139/150 804) This is a minimum value because of the very strict selection of cases Most other authors (38) have found a higher incidence but recently similar and even lower incidences have been reported (1 17 40)

Calculated for four 4 year periods the following incidences were found 1956–59 0 83 (32/38 512) 1960–63 0 85 (34/40 133) 1964–67 1 05 (43/41 143) and 1968–71 0 97 (30/31 016) There are no significant differences between the four incidences (χ^2=1 34 d f =3 0 70< p<0 80)

The age range is 10 to 359 days (median 120 days mean 132 days)

Breast versus artificial feeding

Table 1 shows highly significant differences between cases and controls The general tendency is less breast and more artificial feeding in the cases than in the controls Comparison of prematures (birth weight ≤2 500 g) and matures revealed no significant differences

Table 2 presents the general decline of breast feeding from 1956 to 1971 The case control pattern is uniform throughout the period the relative risk ratio of no longer having exclusively breast feeding being roughly 2 to 3 at any given age and calendar year The relative risk ratio is defined as usual

Table 1. *Percentage (and number) of cases and controls fed breast only, partially breast or artificially only in the second week of life, at one, two, three and four months of age*
At six months of age almost all infants had artificial feeding

	Age of infants					
	Second week		One month		Two months	
	Cases	Controls	Cases	Controls	Cases	Controls
Breast only	60 (74)	79 (412)	36 (39)	60 (303)	11 (11)	34 (173)
Partially breast	20 (24)	13 (68)	17 (18)	19 (94)	11 (11)	21 (105)
Artificially only	20 (25)	8 (40)	47 (51)	21 (108)	77 (75)	45 (225)
χ^2 (d.f.)	23.36 (2)		32.18 (2)		35.18 (2)	
p value	<0.0005		<0.0005		<0.0005	
Dead and unknown	(8)	(4)	(23)	(19)	(34)	(21)

Causes of SIDS in 1969 (3): The sudden death at home of infants between one week and one year, which is unexpected by history, and in which a thorough post mortem examination fails to demonstrate an adequate cause for death.

In Denmark a *police report* is always made within the first hour(s) after an infant has been found dead unexpectedly at home, and an autopsy is requested by the police. The police reports on infants domiciled in the municipality of Copenhagen were reviewed for possible SIDS victims born during the period from 1956 to 1971. Cases whose premortal history included a suspicion of symptoms or illness, which might have caused the death, were excluded. This applies, for instance, to infants with a high temperature, convulsions, or more serious symptoms.

The thorough post mortem examination, including the proposed minimum of investigations (24), was performed by the University Institute of Forensic Medicine in Copenhagen. In 139 cases it failed to demonstrate an adequate cause of death.

The *infant health visitors* in the municipality of Copenhagen are nurses with extra paediatric training who are organized under the supervision of a paediatrician. They keep specially designed record sheets with information about the child's growth, development, and feeding history, in addition to social and perinatal factors (6).

During the period 1956–1972 the infant health visitors

Table 2. *Percentage (and number) of cases and controls fed breast only, partially breast or artificially only in the second week of life, at one, two, three and four months of age*
Divided into four periods of four years each

	Age of infants					
	Second week		One month		Two months	
	Cases	Controls	Cases	Controls	Cases	Controls
1956–1959						
Breast only	94 (29)	88 (112)	52 (14)	65 (81)	20 (5)	37 (46)
Partially breast	–	9 (12)	15 (4)	24 (30)	16 (4)	29 (36)
Artificially only	6 (2)	3 (4)	33 (9)	11 (14)	64 (16)	34 (42)
1960–1963						
Breast only	58 (15)	87 (100)	30 (6)	73 (81)	5 (1)	42 (47)
Partially breast	8 (2)	9 (10)	30 (6)	9 (10)	5 (1)	20 (22)
Artificially only	35 (9)	4 (5)	40 (8)	18 (20)	90 (19)	38 (42)
1964–1967						
Breast only	58 (22)	78 (127)	36 (12)	61 (97)	13 (4)	36 (58)
Partially breast	24 (9)	12 (20)	9 (3)	13 (20)	19 (6)	18 (28)
Artificially only	18 (7)	9 (15)	55 (18)	26 (42)	68 (21)	46 (73)
1968–1971						
Breast only	29 (8)	63 (73)	25 (7)	40 (44)	5 (1)	20 (22)
Partially breast	46 (13)	23 (26)	18 (5)	31 (34)	–	17 (19)
Artificially only	25 (7)	14 (16)	57 (16)	29 (32)	95 (19)	62 (68)

Table 5 *Percentage (and number) of cases plus controls at the age of two three and four months having solids*

Divided into four periods of four years each

	Cases+Controls		
	Two months	Three months	Four months
1956–1959			
Solids	5 (7)	27 (36)	74 (94)
No solids	95 (138)	73 (97)	26 (33)
1960–1963			
Solids	2 (3)	34 (41)	84 (96)
No solids	98 (176)	66 (78)	16 (18)
1964–1967			
Solids	2 (4)	43 (68)	[illegible] (133)
No solids	98 (179)	57 (91)	17 (28)
1968–1971			
Solids	15 (18)	68 (65)	[illegible] (89)
No solids	85 (99)	3 (31)	11 (11)
χ^2 (d f)	9 90 (3)	41 14 (3)	9 75 (3)
p value	<0 0005	<0 0005	0 025–0 050

Solids

Solids (Table 4) include any kind of fruit or vegetable mashes porridge yoghurt etc No significant differences were found between cases and controls

As Table 4 showed no difference between cases and controls the figures are combined in Table 5 to obtain larger numbers in each of the four periods The table demonstrates that solids were introduced significantly earlier in 1968–71 than in previous years

To ascertain whether any cases changed from no solids at all to at least some solids during the last days before death the type of meal described by the health visitor at her last visit was compared with the infant s last meal described in the police report

In half the 104 cases dying at two to six months the infant health visitor had paid a visit to the home within two weeks before death

From one to seven days had elapsed in 23 cases and during this period only one case 60 days old had changed from no solids to solids Seven cases having no solids had not changed ten cases had already been receiving solids and in five cases sufficient information was not available

In the remaining 29 cases eight to fourteen days elapsed between the last visit and death During these days only three cases aged 75 89 and 99 days respectively had changed from no solids to solids while eight cases having no solids had not changed Eight cases were already receiving solids and in ten cases sufficient information was not available

Number of meals per day

In Table 6 the mature and premature infants are kept separate because of significant differences during the second week of life and partly also later on In the case sample as well as in the control sample premature infants received more meals than mature infants There are no significant differences between the cases and controls neither for prematures nor matures

Last meal

In most cases the time of the last meal coincided with the time when last seen alive Thus a minimal period between the last meal and death cannot be estimated But in 38 cases wholly or partially artificially fed at the time of death there was an interval between the last meal and the last time the infant was seen alive This interval was one hour in ten cases two hours in five cases three hours in five cases and four or more hours in 18 cases

DISCUSSION

Breast versus artificial feeding

Like many previous authors (7 27 34 37) we found a lower frequency of breast feeding among SIDS cases as compared with controls (Table 1) Some workers have found no difference (16 19 23 31) Many of the reports are not very detailed mentioning feeding only at discharge from the maternity ward and in some instances at the time of death Only one team (7) followed the type of feeding from month to month using the health visitors records as we did and their results are similar to ours

Table 3 *Percentage (and number) of cases and controls at the age of one two three and four months having fresh cow s milk dilutions of various concentrations calculated as*

$$\frac{milk}{milk+water}\,100\,\%$$

The table comprises all infants who at the given age were receiving such dilutions as part of their diet At six months of age two cases and six controls were receiving 75–80% the remainder 100%

	Age of infants							
	One month		Two months		Three months		Four months	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
50%	79 (44)	70 (96)	12 (8)	7 (16)	2 (1)	2 (4)	(0)	1 ([illegible])
60%	16 (9)	29 (40)	45 (30)	43 (96)	10 (5)	3 (7)	(0)	(0)
65–70%	5 (3)	1 (2)	40 (27)	47 (105)	43 (21)	50 (126)	12 (5)	4 (1[illegible])
75–80%	(0)	(0)	3 (2)	3 (7)	45 (22)	44 (110)	79 (33)	84 ([illegible]60)
100%	(0)	(0)	(0)	0 (1)	(0)	1 (3)	10 (4)	11 (35)
χ^2 (d f)	1 19 (1)		2 0[illegible] (2)		4 92 ([illegible])		0 01 (1)	
p value	0 28		0 36		0 09		0 93	

$$RRR=\frac{\dfrac{\text{No of cases with artificial diet}}{\text{No of controls with artificial diet}}}{\dfrac{\text{No of exclusively breast fed cases}}{\text{No of exclusively breast fed controls}}}$$

Seven of the total number of cases were entirely breast fed throughout life and two were partially breast fed at the time of death In 40 cases data about feeding at the time of death were not available

The age at death for cases who had never been breast fed ($n=24$ range 10–268 days median 126 5 mean 138) and cases who had previously been breast fed ($n=101$ range 23–334 days median 123 mean 131) showed no significant difference when tested by the Mann Whitney test ($t=0\ 627$ d f $=123$ $0\ 50<p<0\ 60$)

Milk formulas

Fresh cow s milk dilutions are prepared by heating cow s milk and water in the concentrations listed in Table 3 to the boiling point often a little sugar is added

In the table the distributions of fresh cow s milk dilutions used show a slight tendency towards more diluted solutions among cases than among controls but these differences are not significant

Powdered milks are in less common use in Denmark The largest numbers are found at two months of age and they show for cases as well as controls that 33% of infants fed artificially wholly or in part had powdered milks We have no information on the concentrations of the powdered milks

Table 4 *Percentage (and number) of cases and controls at the age of two three four and six months having solids*

	Age of infants							
	Two months		Three months		Four months		Six months	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Solids	6 (5)	5 ([illegible]6)	43 (27)	41 (183)	77 (36)	83 (376)	95 (21)	99 (457)
No solids	94 (82)	95 (456)	57 (36)	59 (261)	23 (11)	17 (79)	5 (1)	1 (5)
χ^2 (d f)	0 02 (1)		0 01 (1)		0 69 (1)		0 20 (1)	
p value	0 90		0 91		0 41		0 65	

Four months		Six months	
Cases	Controls	Cases	Controls
(0)	1 (4)	(0)	7 (?9)
(17)	47 (05)	[illegible] (15)	[illegible] (355)
(6)	51 (2_1)	6 (1)	9 (38)
(0)	? (7)	(0)	0 (1)
(0)	(0)	(0)	(0)
3 08 (1)		0 00 (1)	
0 08		>0 95	
18	5 38	5 93	6 02
(67)	(71)	(89)	(85)
(0)	(0)	13 (1)	14 (?)
(6)	43 (6)	75 (6)	71 (10)
(7)	40 (7)	13 (1)	14 (?)
(1)	7 (1)	(0)	(0)
(0)	(0)	(0)	(0)
0 00 (1)		0 78 (1)	
1 00		0 60	
6	5 6	6 10	6 11
(1?)	(?)	(18)	(?)

be expected to live longer than those who have never been breast fed assuming that they are equally exposed to infection Our results failed to demonstrate such a difference in length of life

This problem has been approached in an other way (32) by showing that cases breast fed at the time of death die younger than cases fed artificially at the time of death This tells us nothing about the protective effect of breast feeding but merely establishes that older infants have less breast feeding than younger ones

If artificial feeding is a contributory cause of SIDS the incidence of SIDS would be ex pected to vary with the frequency of artificial feeding Our material shows an overall in crease in artificial feeding through the years (Table 2) but no rise in the incidence of SIDS

The frequency of breast feeding is related to many factors Data as yet unpublished (4) about breast feeding in the municipality of Copenhagen from 1938 to 1972 show a lower frequency of breast feeding of infants born out of wedlock infants of birth order two or more infants of low birth weight infants born to young mothers or to mothers working outside their homes living in poor quality and crowded homes All these factors are known to be connected with SIDS (5 7 16 19 22 37)

In two studies (16 31) cases of SIDS and controls have been matched by social class maternal age birth order and hospital of birth Neither showed any difference between the proportion of cases and of controls who had been breast fed during early infancy An official report (27) has shown that the factor bottle feeding is a significant one only when the general standard of mothering is not in cluded as an explanatory factor in a discrim inant analysis

Many of these materials suggest that pos sibly breast feeding does not help preventing SIDS and that the lower frequency of breast feeding in SIDS cases can be explained by the various concomitant factors mentioned above These factors do not in themselves constitute a cause of death but they may predispose to some unknown condition or conditions leading to SIDS

High solute feeding and overloading

During the past few years attention has been drawn to the danger of high solute feeding of infants (11 13 25 30 33 35) According to some of these reports (33 35) milk formulas are often too concentrated Healthy infants fed artificially on modified cow s milk formulas alone or combined with solid foods have ex hibited significantly higher mean plasma os molality and blood urea than healthy breast fed infants (11 12) Another worker (10) also found the urea concentration to be lower in breast fed than in artificially fed infants but

Table 6 *Percentage (and number) of mature and premature cases and controls fed three four s or seven or more meals a day and average interval (in hours) between meals in the second week life at one two three four and six months of age*

	Age of infants							
	Second week		One month		Two months		Three months	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Mature infants								
3 meals a day	(0)	(0)	(0)	(0)	(0)	0 (1)	(0)	1 (3)
4 meals a day	(0)	0 (1)	(0)	2 (8)	4 (3)	7 (3[illegible])	17 (9)	16 (69)
5 meals a day	22 (17)	23 (103)	46 (44)	36 (175)	76 (59)	70 (335)	79 (41)	77 (336)
6 meals a day	71 (55)	73 (333)	51 (48)	60 (293)	19 (15)	22 (105)	4 ([illegible])	7 ([illegible]9)
$\geq$7 meals a day	8 (6)	4 (17)	3 (3)	2 (9)	1 (1)	1 (3)	(0)	(0)
χ^2 (d f)	0 00 (1)		2 11 (1)		1 39 (2)		0 00 (1)	
p value	>0 95		0 15		0 50		>0 95	
Average interval between meals (hours)	4 13	4 16	4 35	4 31	4 67	4 70	4 98	4 96
Dead and unknown	(27)	(54)	(10)	(23)	(27)	(3[illegible])	(53)	(71)
Premature infants								
3 meals a day	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
4 meals a day	(0)	(0)	(0)	(0)	(0)	(0)	14 ([illegible])	15 ([illegible])
5 meals a day	(0)	(0)	21 (3)	14 (2)	33 (5)	57 (8)	71 (10)	77 (10)
6 meals a day	57 (8)	62 (8)	79 (11)	79 (11)	67 (10)	43 (6)	14 ([illegible])	8 (1)
$\geq$7 meals a day	43 (6)	38 (5)	(0)	7 (1)	(0)	(0)	(0)	(0)
χ^2 (d f)	0 03 (1)		0 00 (1)		0 84 (1)		0 [illegible]1 (1)	
p value	0 87		>0 95		0 36		0 64	
Average interval between meals (hours)	3 76	3 78	4 17	4 07	4 27	4 46	4 86	4 9[illegible]
Dead and unknown	(12)	(3)	(12)	(2)	(11)	([illegible])	(17)	(3)

Calculated from the figures above by dividing 24 hours by number of meals a day followed by averaging $\geq$7 meal a da computed as 7 meals a day

This difference in feeding pattern led to the hypothesis that infants fed on cow s milk are in a hypersensitive state owing to the develop ment of antibodies to milk proteins absorbed from the gut If, during sleep these infants as pirat esime of the stomach contents an acute anaphylactic reaction with fatal outcome can be provoked (28 29) This hypothesis has been supported by guinea pig experiments but the accompanying studies of antibodies in healthy infants and SIDS cases were not quite conclu sive as the two groups were not comparable Subsequent immunological studies (8 9 19 39) have disclosed no case control difference in antibodies or antibody producing cells Fur thermore many authors (19 21 23 37) have found like us that some of the SIDS victims were entirely breast fed throughout Hyper sensitivity cannot explain these deaths unless these infants have received allergens of some kind through the mother s milk

If the anaphylactic hypothesis holds SIDS victims would be expected to die shortly after a meal at least before the stomach is empty None of the infants in our material was ac tually being fed when death occurred Eight een were alive four or more hours after the last meal so that these infants presumably died on an empty stomach

It has been suggested (2 18 20) that SIDS may be the result of an overwhelming infection with which the infant is unable to cope be cause of the immature immune system in the first weeks/months of life Breast fed infants may be passively protected to some extent by receiving antibodies through the mother s milk If this hypothesis is true cases who have been breast fed for at least some time would

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failed to demonstrate any difference in osmolality or sodium concentration

Emery et al (14) examined the vitreous humour of the eye because this should form an isolated pool of fluid retaining after death some of its ante mortem biochemical characteristics, contrary to plasma chemistry which alters rapidly post mortem Among 25 SIDS victims they found in 12 concentrations of sodium and urea indicating hypernatraemia either with or without uraemia Unfortunately no feeding histories were reported They postulate that often hypernatraemia and uraemia are due to the infants being fed concentrated foods and conclude that high solute feeding and water deficiency could be a major factor in SIDS This idea has been supported by a later study (30)

Our epidemiological data give no evidence of SIDS cases getting more concentrated food than controls The most common milk formula fresh cow s milk dilution had been given in the same concentrations to cases and controls (Table 3) The introduction of solids which in view of Davies results (11) quoted above may expose the infant to unsupervised amounts of solutes was found to take place at the same age in cases and controls (Table 4) We found no difference in number of meals per day (Table 6) which shows that cases are not overloaded in the sense that they have fewer and therefore heavier meals Furthermore the fact that so many cases had not been introduced to their first solid meal shortly before death is another suggestion that high solute feeding bears little direct relation to SIDS

The tendency towards earlier introduction of solids through this century (15 36) is clearly seen even in the short period from 1956 to 1971 in our study (Table 5) Should early introduction of solids play a role in the pathogenesis of SIDS the incidence of SIDS should have increased in the same period which it did not

On this background we feel there is no reason to suggest a relationship between high solute load and SIDS

CONCLUSION

We conclude that feeding does not seem to be responsible for the occurrence of SIDS At the same time we should like to stress that like most others we are of the opinion that breast is best but bottle feeding alone should not be accused of causing SIDS Likewise high solute loads should be discouraged but not because of the risk of SIDS

ACKNOWLEDGEMENTS

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THE INCIDENCE OF DIABETES MELLITUS IN SWEDISH CHILDREN 1970–1975

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From the Department of Paediatrics S t Goran s Children s Hospital Stockholm [2]Department of Paediatrics University Hospital Umeå [3]Department of Paediatrics University Hospital Linkoping Department of Paediatrics County Hospital Eskilstuna [5]Department of Paediatrics University Hospital Malmo Department of Paediatrics County Hospital Vanersborg [6]Department of Paediatrics University Hospital Huddinge and [8]Department of Social Medicine University of Umeå Sweden

ABSTRACT Sterky G Holmgren G Gustavson K H Larsson Y Lundmark K M Nilsson K O Samuelson G Thalme B and Wall S (Departments of Paediatrics St Goran s Children s Hospital Stockholm University Hospital Umeå University Hospital Linkoping County Hospital Eskilstuna University Hospital Malmo County Hospital Vanersborg University Hospital Huddinge and Department of Social Medicine University of Umeå Sweden) The incidence of diabetes mellitus in Swedish Children 1970–75 Acta Paediatr Scand 67 139 1978.—We report a retrospective study of diabetic children 0–14 years of age from seven Swedish departments of paediatrics There were 359 new cases in the years 1970–1975 Notification suggested that there was a mean yearly incidence of 19 6 cases per 100 000 with a year to year variation of 10 0–26 4 per 100 000 Consequently about 330 new cases of childhood diabetes would be expected in Sweden every year Incidence varied considerably between different geographical areas The age distribution was bimodal with a main peak at about 12 years and another peak at about 7 years There was some evidence for clustering of new cases in January and the autumn The mean prevalence of childhood diabetes in the seven districts was 1.3 per 1 000

KEY WORDS Juvenile diabetes incidence seasonal variation age at onset

In spite of the unquestionable familial aggregation of juvenile onset diabetes the exact mode of inheritance has not been elucidated The close association of certain HLA types with insulin dependent diabetes suggests a means of identifying predisposed individuals However it is still unknown how the HLA factors confer susceptibility (5) Furthermore the nature of the event(s) precipitating overt diabetes remains unknown

As environmental infectious or toxic factor(s) have been invoked epidemiological studies may give important clues Recent reports suggest marked variations in incidence according to country and geographical location (2 3 4 9) Peak incidence has been observed during winter months (2 4 11) and the distribution of age at onset showed peaks at 5–9 and 11–12 years (2 4)

No studies concerning incidence age at onset or seasonal variations have hitherto been published from Sweden Such a study would seem warranted as health statistics are of good and uniform standard yielding true incidence rates Furthermore certain demographic and socio-economic factors of possible importance are different from those in other countries As no register of all newly diagnosed diabetic children in Sweden is available a retrospective study was undertaken in several regions to provide experience for a countrywide prospective investigation

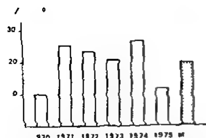


Fig 2 Mean year to year incidence of insulin dependent diabetes in 306 children 0 14 years of age in the years 1970–75

was seen in the northern part of Sweden 28 2 per 100 000 children while in the other areas the range was 17 2–21 0 per 100 000 children

Seasonal variation

Fig 3 shows the seasonal variation of new cases of diabetes each month No statistically significant deviation (0 05<p<0 1) from average was seen The high frequency of new cases seen in January was however statistically significant (0 01<p<0 05) when tested on the hypothesis of a higher frequency of new cases during the winter time There was also a tendency to a smaller peak in September–October

Age incidence

The age distribution at onset in the total material (Fig 4) shows a bimodal distribution with a peak incidence at about 12 years and with a smaller peak at about 7 years These peak incidences deviated significantly from a smooth distribution by age of new cases of diabetes (0 001<p<0 01) and (0 01<p<0 05) respectively The boys had peak incidences at 7 and 13 years respectively and the girls a less pronounced peak at 10 years The mean age at onset for both sexes combined was 8 2 years There was a dramatic fall in incidence at 14 years of age

Family history of diabetes

A history of insulin dependent diabetes in first degree relatives was seen in 11 4% of the patients

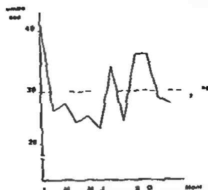


Fig 3 Seasonal variation of childhood onset diabetes in 359 children 0–14 years of age in the years 1970–75

Prevalence

The mean prevalence of insulin dependent diabetes was 1 3 per 1000 children with an area to area variation of 1 0 to 1 9 (Table 2) Calculated from the total Swedish childhood population 0–14 years on the 1st January 1975 1 693 135 the number of diabetic children would be around 2 400

Great variation was seen in the prevalence rates with highest rates in the northern part of Sweden the lowest frequencies were seen in the densely populated areas in the southern part of Sweden

DISCUSSION

In previous studies (2 3 4) a male excess of diabetic children has been reported which was not seen in the present study

Table 2 *The prevalence of insulin dependent diabetic children 0–14 years of age on the 31 December 1975*

Hospital area no	Total no of children 0–14 years of age	Diabetic children 0–14 years of age	Prevalence per 1 000
1	32 377	60	1 9
2	47 063	69	1 5
3	24 986	37	1 5
4	40 413	48	1 2
5	36 193	50	1 4
6	–	–	–
7	77 924	78	1 0
Total	258 956	342	1 3 (mean)

See text

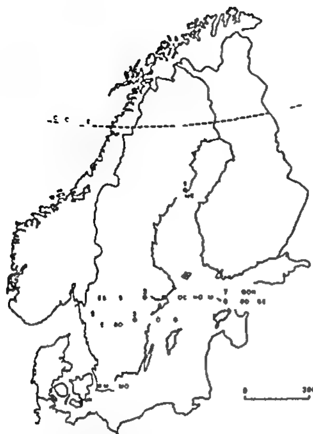


Fig 1 Map of Scandinavia showing the different hospital areas of the present study

MATERIAL AND METHODS

In Sweden all children aged 0–14 years are referred to paediatric departments in hospitals when diabetes is suspected (12) In the present study seven departments of paediatrics from different parts of the country participated Three of the hospitals (Nos 4 6 and 7 in Fig 1 and Tables 1 and 2) are situated in densely populated areas with a total population exceeding 200 000 individuals the other four are in more sparsely populated areas The total number of children 0–14 99 years of age in the areas studied was 351 460 i e constituting 20% of these age groups in all Sweden

Data were collected using a standardized form recording the patient s date of birth sex hospital number date at onset of diabetes and whether insulin dependent diabetes was present in sibs or parents Date at diagnosis was defined as the day when the first insulin injection was given Data were analysed by computer

There were 359 patients 0–14 years of age with an onset of diabetes in the years 1970–75 Great efforts were made to ensure that the patient at the date of diagnosis really lived in the geographical area in question

Yearly incidence of diabetes was calculated for six areas For the study of diabetes prevalence data were notified from six hospitals concerning all insulin dependent diabetic children 0–14 years of age on the 31 December 1975 Hospital No 6 had to be excluded in these calculations as it was not opened until May 1974

Seasonal variations were tested overall as deviations from a uniform distribution by means of standardized chi square tests As specific hypotheses (? 4 11) about seasonality and age at onset were stated in advance we also utilized normalized tests of differences between proportions

RESULTS

Sex distribution

Of the 359 children with insulin dependent diabetes 184 were boys and 175 girls The sex ratio 1 05 is not significantly different from the overall sex distribution in this age group in Sweden

Incidence

The mean yearly incidence of diabetes in the years 1970–75 was 19 6 per 100 000 children (Table 1) Since our material constitutes 16 per cent (hospital No 6 excluded) of the total Swedish population in ages 0–14 years and included individuals from different parts of the country it is possible to calculate the total number of new cases of childhood diabetes in Sweden each year This number is approximately 330

Year to year variations in incidence were considerable and ranged from 10 0 to 26 4 per 100 000 children in the total material (Fig 2) with even greater variations within each area (Table 1) The highest mean yearly incidence

Table 1 *The yearly incidence of insulin dependent diabetes in children 0–14 years of age in 1970–75 in seven hospital areas*

Hospital area no	The total no of children 0–14 years of age Mean of years 1970–75	Diabetic children 0–14 years of age	Mean yearly incidence 1970–75 per 100 000	Year to year range 1970–75
1	31 339	53	28 2	9 6–38 3
2	48 586	61	21 0	2 4–37 0
3	24 928	28	18 6	4 0–40 0
4	45 400	48	17 7	6 6–24 [illegible]
5	32 793	36	18 3	9 1–24 4
6	70 414	53	–	–
7	78 000	80	17 2	9 0–29 5
Total	331 460	359	19 6 (mean)	2 4–40 0

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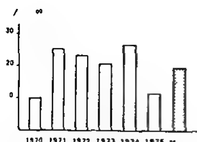


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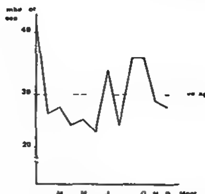


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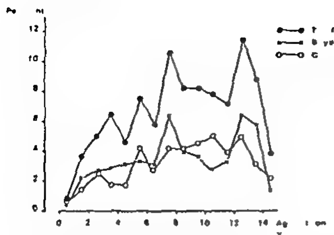


Fig 4 Age incidence of insulin dependent diabetes in 184 boys and 175 girls as well as in the total material

The incidence rates for diabetic children 0–14 years in the present study (19 6/100 000) are higher than those seen in northern Norway (3) (8 0/100 000) Denmark (4) (13 7/100 000) and Great Britain (2) (8 0/100 000) but lower than in Finland (9) (29/100 000 in the age group 0–19 years) In Finland (9) the lowest rates were seen in the northern part of the country (26/100 000) while in Sweden the highest rates were seen in the northern part (28 2/100 000) Nevertheless the higher frequencies of diabetes seen in the northern part of Sweden might partly be due to gene flow from the Finnish population and gene enrichment through inbreeding (1)

The higher incidence rates of new diabetics in the winter time with a peak incidence in January in the present study has previously been observed in Great Britain (2) Denmark (4) and Minnesota USA (11) These observations might reflect an influence of environmental factors e g viral infections and there has been reported a remarkable similarity between the seasonal pattern in the incidence of juvenile diabetes and the seasonal pattern of infections of childhood (6) Most of these infections are minor respiratory illnesses and it seems unlikely that they would cause diabetes though they might constitute a stress factor sufficient to precipitate overt diabetes in patients who had previously sustained islet cell damage It is still possible however that virus infections specifically damage pancreatic islet cells

In several studies concerning age incidence of childhood diabetes a bimodal distribution is seen In Great Britain (2) peak incidences were observed at about 5 and 11 years of age respectively In Denmark the peak incidences occurred at 7–9 and 12 years respectively (4) In the present study a main peak was seen at 12 years of age and a smaller peak at 7 years There were some shifts in the sex ratios which as yet do not allow interpretation The bimodal distribution of age at onset of diabetes might arise from two groups with different aetiologies (5) The first peak is associated with starting school in Great Britain at the age of 5 years in Scandinavia at the age of 7 years The aetiology might be viral infections or non specific stress factors The second peak at onset of diabetes at about 12 years of age has been suggested to be associated with pubertal changes or with spurts in the growth rate In Sweden peak height velocity occurs at 11 9 years in girls and 14 0 years in boys and menarche occurs at 13 years of age (8) Direct relationships must await analysis of individual growth rates and onset of diabetes

In the present study 11 4% of the children had a history of insulin dependent diabetes in a first degree relative The material was too small for analysis in respect of age and seasonal incidence

The prevalence of diabetes in children 0–14 years in the present study was 1 3 per 1000 This rate is similar to that observed in Stockholm 1960 by Sterky (12) in school children aged 7–14 years—1 4 per 1000 The highest prevalence rate in the present study (1 9/1000) was seen in northern Sweden (area No 1 Fig 1) In a previous study (7) performed in this area on children 0–15 years of age the rate was 2 2 per 1000 In Finland Koivisto et al (9) have reported a prevalence of insulin dependent diabetes in children 0–19 years of 2 23 per 1000 When comparing the prevalence to incidence ratios of childhood diabetes in some European countries Lestradet et al (10) ob-

served a striking difference between data given for Great Britain and the others As pointed out by Bloom et al (2) this may be due to a less accurate incidence rate

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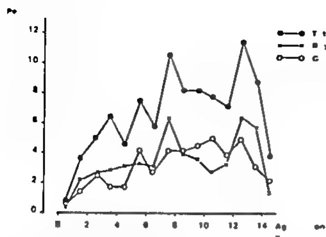


Fig 4 Age incidence of insulin dependent diabetes in 184 boys and 175 girls as well as in the total material

The incidence rates for diabetic children 0–14 years in the present study (19 6/100 000) are higher than those seen in northern Norway (3) (8 0/100 000) Denmark (4) (13 7/100 000) and Great Britain (2) (8 0/100 000) but lower than in Finland (9) (29/100 000 in the age group 0–19 years) In Finland (9) the lowest rates were seen in the northern part of the country (26/100 000) while in Sweden the highest rates were seen in the northern part (28 2/100 000) Nevertheless the higher frequencies of diabetes seen in the northern part of Sweden might partly be due to gene flow from the Finnish population and gene enrichment through inbreeding (1)

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CESSATION OF THERAPY IN CHILDHOOD LEUKEMIA

A Survey of 160 Cases from the Nordic Countries

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ABSTRACT Moe P J (Department of Paediatrics University of Tromsø Norway) Cessation of therapy in childhood leukemia Report of a Nordic material consisting of 160 cases Acta Paediatr Scand 67 145 1978.—A survey is presented of 160 children from the Nordic countries who had their antileukemic therapy discontinued prior to November 1976 Twenty seven of the 160 cases (17 %) had suffered a relapse before May 1977 Sixty nine cases had their therapy stopped in the first ten months of 1976 All cases have been reported as acute lymphocytic leukemia Different types of therapy schedules have been used Thirty five cases in sustained remission for more than 3 years without cessation of therapy are also included in the report seventeen of whom had relapsed while still on therapy Central nervous system or testicular relapse occurred in 21 of the total 44 cases who relapsed after three or more years of continuous remission and whether they were on therapy or not

KEY WORDS Childhood leukemia therapy Nordic countries CNS-involvement testicular disease sanctuaries

The introduction of total therapy in acute lymphocytic leukemia (ALL) in childhood is perhaps one of the most important medical developments in the last decade It appears that therapy is not necessarily a lifelong procedure in all cases For instance between 1972 and 1975 we stopped therapy in 13 of 43 cases of ALL whose primary therapy was initiated by us between 1963–1972 (7 9) In May 1977 all 13 cases were still in their primary remission The total number of our cases with childhood leukemia were however small Actually there are only about 42 new cases of childhood leukemia every year in Norway (7) The purpose of this study has been to try to evaluate the effect of cessation of therapy in childhood leukemia in the five Nordic countries

MATERIALS AND METHODS

The five Nordic countries Denmark Iceland Finland Norway and Sweden have about 22 million inhabitants Each year there are about 200 new cases of leukemia in these countries in individuals below 15 years of age (7 and Table 1) Results of therapy in all new cases of childhood leukemia during the period 1963–1974 in Norway have been reported (7) In 1975 the university and regional departments of pediatrics in the other Nordic countries were also contacted in order to try to identify cases of leukemia in primary remission for 3 years or more particularly the cases off therapy in their regions In 1976–1977 a final inquiry was sent to these and the other departments of pediatrics in the Nordic countries

The following questions were made to a total of 108 departments of pediatrics and to 4 other departments of the Nordic countries treating children with leukemia

1 Have you discontinued antileukemic therapy before November 1976 in any child in prolonged remission Birth dates sex time of diagnosis and time of discontinuation of therapy

2 Have any of the cases relapsed time and site of relapse

3 Have you any other cases who has been in primary remission for more than 3 years without cessation of therapy Have any of them relapsed time and site of relapse

Answers were obtained from 105 of the 112 asked departments including all university and regional departments The possibility exists that information has not been obtained on a few cases

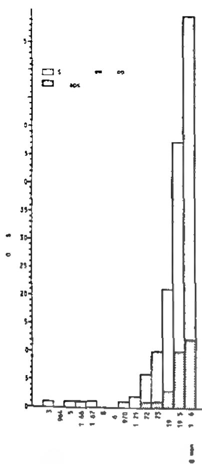


Fig 2 Year of cessation of therapy

It may be seen that therapy has been discontinued in a rapidly increasing number of cases after 1973 During the first 10 months of 1976 therapy was stopped in a total of 69 cases The relapse rate has been about the same for each year the observation time is less than one year in 30 cases diagnosed in 1973

The number of cases off therapy and the number of relapsers in the individual Nordic countries are shown in Table 1 The number of cases off therapy in relation to the number of diagnosed cases in the respective countries were somewhat lower in Denmark This is however due to the fact that one seems to have been a little more reluctant to stop therapy in Denmark after 3 years of sustained re- sion than in the other Nordic countries

Table 2 *Site of relapse in the 27 cases who relapsed after cessation of therapy and in 17 cases relapsing while on therapy for more than 3 years*

Site of relapse	Cases off therapy	Cases on therapy	Total
Central nervous system	[illegible]	5	11
Testes	6	4	10
Lung	1	[illegible]	1
Bone marrow	12	6	18
Not specified	4	2	4
Total cases	7	17	44

(Table 1) There is not a big difference between the five countries if all cases in sustained remission for more than 3 years and still in remission are included The possibility that not every single case still in prolonged remission have been traced must also be born in mind

The relapse rate after cessation of therapy has been particularly high in Sweden (24%) and low in Finland (8%) but the total number of cases off therapy in the individual countries were low and the difference may therefore be due to chance Table 2 shows the site of relapse in the 27 cases who relapsed while off therapy and in the 17 relapsing while on therapy They had been in remission for at least 3 years Central nervous system or testicular involvement occurred whether the patients were on therapy or not

Table 3 *Age at time of the diagnosis in the 160 cases whose therapy had been discontinued compared to usual age distribution of leukemia*

Age	No of cases	%	Age distribution in % of 499 Norwegian cases
0–11 months	3	1 9	5 0
1 –23 months	10	6 2	9 4
2– 9 years	130	81 3	69 4
10–14 years	17	10 6	16 ?
Total no of cases	160	100	100

Table 1 *Surveys of the materials from the individual Nordic countries*

Country	No of cases off therapy	Relapse after cessation	Primary remission >3 yrs without cessation		No of cases per year (11)
			Total	Relapse	
Denmark	25	5	15	4	45 (1946–57)
Finland	37	3	8	5	47 (1953–73)
Iceland	1	0	0	0	2
Norway	43	6	2	2	42 (1963–73)
Sweden	54	13	10	6	60 (1969–74)
Total	160	27	35	17	196

A follow up has been made in April 1977 half a year after therapy was discontinued in the last case in the registration period Information was obtained in all 160 cases off therapy Some of the cases had not been checked for a while since they had been off therapy for a long time It is however unlikely that they had relapsed

Numerous different therapeutic programmes have been used in the five Nordic countries in the past and it is beyond the scope of this investigation to try to collect information on all of them

CNS prophylaxis was started in Bergen in 1971 (8) but has only been in routine use in the Nordic countries since 1972–73 Different types of CNS prophylaxis have been used including both Methotrexate intrathecally alone and a combination of Methotrexate intrathecally with cranial and craniospinal irradiation

RESULTS

A total of 160 patients with acute leukemia whose therapy had been discontinued before November 1976 have been identified in the five Nordic countries 73 were boys They were all below the age of 15 years at the time of diagnosis Twenty seven of the 160 cases had relapsed before May 1977 Thirty five cases (17 boys) on therapy treated for more than 3 years while still in primary remission were also reported Seventeen had relapsed

All cases have been diagnosed as acute lymphocytic leukemia or undetermined type Reservation as to type of leukemia has to be made as it has not been possible for the author to check the blood and bone marrow smears from all the different places No definite case of acute myelogenous leukemia off therapy after remission for 3 years have been reported Fig 1 shows the year of diagnosis in the 160 cases including the 27 relapsers Only 21 cases diagnosed in the Nordic countries before 1968 had their therapy discontinued From 1968 there has been a marked increase in the annual number of new cases off therapy, 43 cases diagnosed in 1973 had their therapy stopped before November 1976 This means that of the estimated new cases diagnosed as childhood leukemia in the Nordic countries in 1973 approximately 20% had their therapy discontinued before November 1976 One case of relapse was seen among the 16 cases off therapy diagnosed in the period 1959–1966 (Fig 1) The relapse rate has been about 18% in the following years The year of cessation of anti leukemic therapy in the 160 cases is shown in Fig 2 Therapy was stopped in only 7 children altogether before 1972 in the Nordic countries

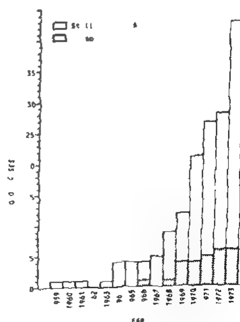


Fig 1 Year of diagnosis in cases off therapy

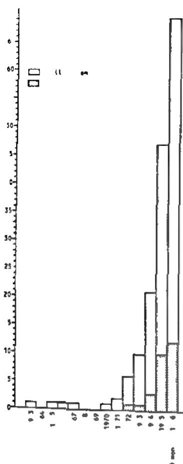


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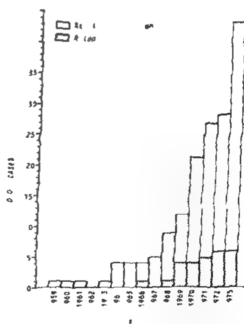


Fig 1 Year of diagnosis in cases off therapy

Rosen & Nowack (11) even reported bone marrow relapse in three of 12 children after discontinuation of therapy following 5 years of maintenance therapy and continuous remission Our results support the concept that it is not necessary to continue therapy for more than 3 years provided that there is no evidence of disease The risk of relapse after cessation of therapy is greatest in the first year

Our therapy has been even more aggressive during the last 2 years after the introduction of a consolidation phase including high dose methotrexate (10) The question is actually whether [redacted] stop therapy after less than 3 years of remission The St Jude group stopped therapy after 2–3 years of continuous remission in their 132 cases

Simone et al reported (12) that the relapse rate was considerably higher in children not receiving CNS prophylaxis Our data confirm that conclusion CNS involvement has been a problem in long term survivors in the Nordic countries Total therapy including CNS prophylaxis was however given to most cases from 1972–1973 This is probably the main reason why there is a much higher proportion of children in prolonged remission from 1972–1973 in the Nordic countries than from previous years Therapy was actually discontinued in 69 cases during the first 10 months of 1976 Thirty six of them had only been treated for 3 years

CNS and testicular involvement occurred in the Nordic material regardless of cessation or continuation of therapy Extensive use of CNS prophylaxis will probably reduce the number of cases of CNS involvement Conventional therapy does not seem to prevent testicular infiltration which occurs in about 16% of males (4)

For years it has been known that the prognosis of acute lymphocytic leukemia is worse in infants and [redacted] children above the age of 10 years Our data confirm the poorer prognosis particularly in infants below the age of one year

ACKNOWLEDGEMENTS

I wish to express my most sincere thanks to colleagues of the Nordic hospitals who kindly provided information on childhood leukemia in this study particularly to the 34 departments contributing information on the 160 cases whose therapy had been discontinued

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Table 4 *Length of therapy in the 160 cases*

Length of therapy (y)	No of cases without relapse	Relapse
<3	2	0
3	64	12
3½	8	1
4	16	2
4½	5	3
5	19	5
>5	19	4
Total	133	27

Twenty three of the 27 relapsed within one year after cessation of therapy three after 1–2 years and one after more than 2½ years

The age at time of diagnosis in the 160 cases is shown in Table 3 The youngest patient was 2 months at the time of diagnosis Thirteen children were below 2 and 7 above 10 years of age The age distribution in 499 Norwegian cases of childhood leukemia are presented for comparison The table shows that the prognosis for children under 2 and above 10 years are not as good as for those between 2–10 years of age All but two cases had been treated for 3 years or more Of the 27 cases who relapsed after treatment 46% had their therapy stopped after less than 4 years Of the total 160 cases 55% had been treated for the same length of time (Table 4) Four of the reported 160 cases were in their second remission while therapy was discontinued after at least 3 years of continuous remission so far one of them has relapsed Seventeen children relapsed while still on therapy which had lasted for more than 3 years (Table 2)

DISCUSSION

The ultimate goal of treatment of children with acute leukemia is permanent remission of the disease Leukemia free survival after cessation of therapy is relevant to potential cure and there is no doubt that leukemia free survival has improved to such a degree that discussion of cure is relevant

The optimal duration of maintenance therapy is not known The hazards of long term therapy must be weighed against the potential benefit Burchenal in 1968 (2) suggested that therapy should be stopped if the patient survived 7 years and had been free of leukemia for the past 4 years Vowels & Willoughby (13) stopped therapy when 96 months of remission had been reached Krivit et al (3) discontinued therapy in eight of fifteen children who had been in complete clinical and bone marrow remission for a minimum of 2½ years and continued therapy in the other seven During the ensuing 2 years there was no significant difference in relapse rate in the two groups On the other hand it is evident that 6 months maintenance therapy was too short (5 6)

Simone et al (12) reported in 1972 that therapy was stopped after 2 or more years of complete remission in 42 patients 9 of them relapsed Six of the 9 had however not received CNS prophylaxis

Cessation of therapy even in long term survivors was uncommon in the Nordic countries until 1972 It has been possible to trace only seven such cases altogether This may partly be due to the fact that long lasting remission was rare in the Nordic countries until the last 5 years and partly because continuous therapy was considered a life long supporting necessity in childhood leukemia There has however been a change in attitude In 1975–1976 it was generally accepted to stop therapy after 3 years or more of continuous remission Our relapse rate after cessation of therapy (17%) is about the same as reported by Aur et al in 1974 (1) Twenty one of the 132 (16%) cases who had their therapy stopped at the St Jude hospital had developed recurrent leukemia 39 cases had been followed for less than one year post treatment

The post treatment relapse rate after 3 years of therapy seems in our study to be about the same as in the cases who had their therapy stopped after four or more years of therapy Furthermore relapse was seen in a considerable number of cases while they were on therapy for more than 3 years

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HYPOPHYSO GONADAL FUNCTION IN THE DIABETIC CHILD

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ABSTRACT Cicognani A Zappulla F Bernardi F Capelli M Mazzanti L. Turchi S Radetti G Pirazzoli P and Cacciari E (Department of Paediatrics University of Bologna Bologna Italy) Hypophyso-gonadal function in the diabetic child Acta Paediatr Scand 67 151 1978 —14 diabetic boys (five with a family history of diabetes and nine without) and 29 short normal boys were studied A gonadal function test (2 000 IU of hCG i m for 3 days and plasma testosterone assay before and after the hCG administration) as well as an LH RH test (50 μg i v) were carried out While basal testosterone level turned out to be similar in the two groups of children it was significantly lower ($p<0.01$) after hCG than the mean value of the control group This difference was mainly observed in those patients with a family history of diabetes In the diabetic children basal LH level was normal and the pituitary LH reserve was lower than in the control group Both basal FSH level and FSH pituitary reserve were lower than in normal children These data show that an alteration in the hypothalamus–pituitary–gonadal function is already evident in the diabetic child

KEY WORDS Hypophyso–gonadal function diabetic child LH RH test hCG test

Alterations in the sexual function and in the histology of the testes are common in the diabetic man (7 11 12 16 18) However there are few and contrasting data on the function of the hypothalamus–pituitary–gonadal axis in these patients (6 8 13) Since the use of dynamic tests enables us to reveal possible pituitary gonadal alterations even in the pre pubertal stage (1 3 4 9 15 17 22) we studied the hypothalamus–pituitary–gonadal axis in the diabetic child

MATERIALS AND METHODS

Twenty nine "short normal" boys (chronological age ranging from 4 ⁴/₂ to 1⁷ /₁₀ years mean 8 / bone age ranged from 4 / to 1⁷ years mean 8 / ₂) and 14 diabetic boys (chronological age ranging from 5 to 1 years mean 8 / bone age ranging from 5 / ₂ to 1⁷ years mean 8 / ₂) were studied In the diabetic group the family history of diabetes was taken into consideration and the boys with (no 5) or without (no 9) this genetic characteristic were evaluated separately Children were considered as having a family history of diabetes if at least one sibling parent grandparent aunt or uncle was affected by an insulin-dependent diabetes In all the cases examined the difference between the chronological and the bone age never exceeded 8 months All the children examined fell into the prepubertal stage of sexual development i e the first stage according to Tanner (21) Bone age was determined according to the tables of Greulich & Pyle (10) All the diabetic children received insulin (Novolenta) administered with one i m injection in the morning before breakfast The children were on a balanced diet according to their ages that took into account the individual patients tastes and contained 40–45% of carbohydrates

All the children with their parents permission underwent both a gonadal function and an LH RH test The gonadal function test was carried out as follows 2 000 IU of hCG were administered every day for 3 days at 9 a m on the first 2 days and at 6 a m on the third day Immediately before the beginning and at the end of the test (at 9 a m) a blood sample was drawn for the assaying of plasma testosterone The LH RH test was performed at 9 a m after an overnight fast using intravenous injection of 50 μg of synthetic LH RH (Farbewerke Hoechst AG) Venous blood for the evaluation of LH and FSH was collected at times 0 15 30 45 60 and 90 min

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HYPOPHYSO GONADAL FUNCTION IN THE DIABETIC CHILD

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ABSTRACT Cicognani A Zappulla F Bernardi F Capelli M Mazzanti L Turchi S Radetti G Pirazzoli P and Cacciari E (Department of Paediatrics University of Bologna Bologna Italy) Hypophyso-gonadal function in the diabetic child Acta Paediatr Scand 67 151 1978 —14 diabetic boys (five with a family history of diabetes and nine without) and 29 short normal boys were studied A gonadal function test (2 000 IU of hCG i m for 3 days and plasma testosterone assay before and after the hCG administration) as well as an LH RH test (50 μg i v) were carried out While basal testosterone level turned out to be similar in the two groups of children it was significantly lower ($p<0.01$) after hCG than the mean value of the control group This difference was mainly observed in those patients with a family history of diabetes In the diabetic children basal LH level was normal and the pituitary LH reserve was lower than in the control group Both basal FSH level and FSH pituitary reserve were lower than in normal children These data show that an alteration in the hypothalamus–pituitary–gonadal function is already evident in the diabetic child

KEY WORDS Hypophyso–gonadal function diabetic child LH RH test hCG test

Alterations in the sexual function and in the histology of the testes are common in the diabetic man (7 11 12 16 18) However there are few and contrasting data on the function of the hypothalamus–pituitary–gonadal axis in these patients (6 8 13) Since the use of dynamic tests enables us to reveal possible pituitary gonadal alterations even in the prepubertal stage (1 3 4 9 15 17 22) we studied the hypothalamus–pituitary–gonadal axis in the diabetic child

MATERIALS AND METHODS

Twenty nine short normal boys (chronological age ranging from 4 / to 12 / years mean 8 / bone age ranged from 4 / to 1 years mean 8 /) and 14 diabetic boys (chronological age ranging from 5 to 12 years mean 8 / bone age ranging from 5 / to 12 years mean 8 /) were studied In the diabetic group the family history of diabetes was taken into consideration and the boys with (no 5) or without (no 9) this genetic characteristic were evaluated separately Children were considered as having a family history of diabetes if at least one sibling parent grandparent aunt or uncle was affected by an insulin-dependent diabetes In all the cases examined the difference between the chronological and the bone age never exceeded 8 months All the children examined fell into the prepubertal stage of sexual development i e the first stage according to Tanner (21) Bone age was determined according to the tables of Greulich & Pyle (10) All the diabetic children received insulin (Novolenta) administered with one i m injection in the morning before breakfast The children were on a balanced diet according to their ages that took into account the individual patients tastes and contained 40–45% of carbohydrates

All the children with their parents permission underwent both a gonadal function and an LH RH test The gonadal function test was carried out as follows 2000 IU of hCG were administered every day for 3 days at 9 a m on the first 2 days and at 6 a m on the third day Immediately before the beginning and at the end of the test (at 9 a m) a blood sample was drawn for the assaying of plasma testosterone The LH RH test was performed at 9 a m after an overnight fast using intravenous injection of 50 μg of synthetic LH RH (Farbewerke Hoechst AG) Venous blood for the evaluation of LH and FSH was collected at times 0 15 30 45 60 and 90 min

Table 3 *Peak maximum increase and area of the LH and FSH curves in 29 normal and in 14 diabetic boys (mean ±S E M)*

		Peak (mIU/ml)		Maximum increase (mIU/ml)		Area of the curve	
		LH	FSH	LH	FSH	LH	FSH
Normal children	29	4 37 ±0 57	6 33 ±0 56	2 56 ±0 51	4 07 ±0 51	20 64 ±2 28	27 89 ±[illegible] 78
Diabetic children	14	2 76 ±0 35	3 74[b]	1 36 ±0 33	2 [illegible]9 ±0 [illegible]6	13 16 ±1 61	16 96[a] ±1 90
with familial diabetes	5	2 56 ±0 60	3 80 ±0 59	0 92 ±0 16	[illegible] 56 ±0 37	13 [illegible]4 ±3 05	17 54 ±2 58
without familial diabetes	9	2 87 ±0 45	3 38 ±0 55	1 61 ±0 30	2 16 ±0 36	13 12 ±3 99	17 07 ±[illegible] 37

$p<0.05$ (for the difference with normal boys)
• $p<0.01$ (for the difference with normal boys)

significant ($p<0.05$) only for the whole group of diabetics at the 45th and 60th minute (Table 2) There were no differences between the two sub groups The mean FSH curve was constantly lower in the whole group of diabetic children than in the control group and this difference was significant (Table 2) No significant differences were observed between the two subgroups of diabetics

The pituitary gonadotropin reserve that was studied by evaluating the peak the maximum increase and the area of the curve (2) (Table 3) revealed significant differences between normal and diabetic patients As far as LH is concerned the difference was weakly significant ($p<0.05$) only for the peak and for the area in the intire group of diabetic children

FSH (mIU/ml)					
0	15	30	45	60	90
13 ±0 [illegible]	3 54 ±0 [illegible]8	4 71 ±0 39	5 49 ±0 44	5 28 ±0 46	5 16 ±0 61
1 4 ±0 [illegible]	[illegible] [illegible]7[a] ±0 [illegible]9	[illegible] 83 ±0 30	3 [illegible]4 ±0 4	3 16 ±0 36	3 1[illegible] ±0 40
1 [illegible]8 ±0 [illegible]6	[illegible] [illegible] ±0 40	[illegible]86 ±0 40	3 48 ±0 57	3 48 ±0 56	3 08 ±0 45
1 58 ±0 30	[illegible]9 ±0 41	[illegible] 81 ±0 4	3 10 ±0 58	[illegible] 99 ±0 47	3 14 ±0 59

As to FSH the difference was weakly significant ($p<0.05$) for the maximum increase and significant ($p<0.01$) for the peak and the area in the entire group of diabetics No significant differences were found between the two sub groups

Both in the normal children and in the diabetics no correlation between bone age and basal LH values or pituitary LH reserve was found In the normal children a positive correlation was found ($r=+0.485$ $p<0.01$) between bone age and LH area No correlation was observed in the two groups of patients between basal or even post hCG testosterone and basal FSH or pituitary FSH reserve As far as LH is concerned a positive correlation was found in the diabetic group between post hCG testosterone and LH peak ($r=+0.625$ $p<0.05$) No correlation was found between duration of the diabetic disease and testosterone or gonadotropin behaviour

DISCUSSION

In the diabetic boys the testosterone response to hCG was significantly weaker than that obtained in normal boys of the same age In our study this difference turned out to be mainly ascribable to the few cases (five) with a family history of diabetes In fact in patients without a familial diabetic connection the re

Table 1 *Basal and post hCG plasma testosterone in 29 normal boys and in 14 diabetic boys (5 with diabetes in the family and 9 without)*

	No	Basal testosterone (ng/100 ml)		Post hCG testosterone (ng/100 ml)	
		Mean	S E M	Mean	S E M
Normal children	29	16 51	1 99	106 54	9 89
Diabetic children	14	17 64	3 82	62 46[a]	10 17
with familial diabetes	5	13 80	2 19	45 70[b]	11 78
without familial diabetes	9	19 78	5 82	72 06	13 81

[a] $p<0\ 01$ (for the difference with normal boys)
[b] $p<0\ 05$ (for the difference with normal boys)

Plasma testosterone was determined according to the radioimmunoassay method of Collins et al (5) The antiserum used was obtained from rabbits pre treated with an anti tubercular vaccine as described previously (1) The antiserum was used at a dilution of 1 30000 The sensitivity of the method was 0 5 ng/100 ml The variation coefficient of duplicate samples was ±4 8% Serum LH and FSH were evaluated according to the double antibody radioimmunoassay method of Reuter et al (14) using human pituitary LH and FSH The radioimmunological equivalent of LH is 2 150 IU as compared with the 68/40 reference preparation of the National Institute for Medical Research (MRC) Mill Hill London For FSH this is 2 800 IU as compared with the 68/39 reference preparation of the MRC The results are expressed as mIU/ml MRC reference preparation The sensitivity of the method was 0 5 mIU/ml both for LH and FSH The variation coefficients of duplicate samples were ±7 4% and ±8 5% for LH and FSH respectively

As far as the diabetics are concerned the correlations were evaluated given the low number of patients without taking the family history of diabetes into consideration

RESULTS

Basal testosterone did not reveal any significant differences between the various groups (Table 1) After hCG, the mean testosterone level was significantly lower ($p<0\ 01$) in the diabetic children taken as a single group The difference was also weakly significant ($p<0\ 05$) for the sub group with a family history of diabetes whereas it was not significant for the sub group without diabetes in the family history The response to hCG did not show any difference between the two sub groups Both in the normal children ($r=+0\ 479$ $p<0\ 01$) and in the diabetics taken as a single group ($r=+0\ 703$, $p<0\ 01$) there was a significant positive correlation between bone age and basal testosterone level The positive correlation ($r=+0\ 381$ $p<0\ 05$) between bone age and post hCG testosterone level observed in normal children was not found in the diabetic patients

The mean LH curve (Table 2) was constantly lower in the diabetics than in normal children but the difference was weakly

Table 2 *LH and FSH values (mean ±S E M) in normal and diabetic children who underwent the LH RH test (50 μg i v)*

	No	LH (mIU/ml)					
		0	*15*	*30*	*45*	*60*	*90*
Normal children	29	1 81 ±0 15	3 68 ±0 50	4 00 ±0 51	3 72 ±0 37	3 55 ±0 45	2 97 ±0 33
Diabetic children	14	1 39 ±0 25	2 40 ±0 29	2 55 ±0 33	2 31 ±0 31	2 16 ±0 32	1 98 ±0 24
with familial diabetes	5	1 64 ±0 49	2 36 ±0 58	2 46 ±0 60	2 28 ±0 52	2 16 ±0 54	2 16 ±0 41
without familial diabetes	9	1 26 ±0 29	2 42 ±0 35	2 60 ±0 42	2 33 ±0 40	2 17 ±0 43	1 87 ±0 30

$p<0\ 05$ (for the difference with normal boys)
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0	15	30	45	60	90
13 ±0 2	3 54 ±0 28	4 71 ±0 39	5 49 ±0 44	5 78 ±0 46	5 16 ±0 61
1 4 ±0 1	77 ±0 29	83 ±0 30	3 24* ±0 47	3 16* ±0 36	3 17 ±0 40
1 8 ±0 6	4 ±0 40	86 ±0 40	3 48 ±0 57	3 48 ±0 56	3 08 ±0 45
1 58 ±0 30	79 ±0 41	81 ±0 4	3 10 ±0 58	2 99 ±0 47	3 14 ±0 59

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DISCUSSION

In the diabetic boys the testosterone response to hCG was significantly weaker than that obtained in normal boys of the same age In our study this difference turned out to be mainly ascribable to the few cases (five) with a family history of diabetes In fact in patients without a familial diabetic connection the re

sponse to hCG (though weaker) did not differ significantly from that of the control subjects In patients with a familial diabetic connection on the contrary, the response was significantly lower than that observed in the normal children and is definitely lower (although not significantly) than the response obtained from the other diabetic sub group (Table I)

Studies carried out on adult diabetics (8 11) indicated a normal basal testosterone level and a response to hCG significantly lower than normal These investigations however did not take into consideration any possible difference between patients with and without a family history of diabetes

In our patients a significant alteration in the endocrine–gonadal function was associated with a normal basal LH level and a decrease in the pituitary LH reserve together with a lower FSH basal level and a decrease in the FSH pituitary reserve (Tables 2 and 3) In the diabetic adult with or without impotence while Rastogi et al (13) did not demonstrate any significant alteration in the pituitary LH and FSH reserve Distiller et al (6) found a diminished gonadotropin reserve

Not only do our data show that the diabetic patient already displays alterations in the function of the hypothalamus–pituitary–gonadal axis in childhood but they also indicate the possibility that the genetic factor i e the diabetic familiality may play a fundamental role in causing it Although only a few cases were examined we still feel it interesting that considering the diabetics with and without diabetic familiality separately the response to hCG is significantly different from the normal subjects only in the former patients

We can assume that vascular alterations are at least partly responsible for the disorder both at gonadal and hypothalamus–pituitary level We should remember, however that the endocrine behaviour of the gonads might be the result of the diminished FSH release It has actually been demonstrated in hypophysectomized rats that FSH plays an important role in controlling the testosterone secretion (20), and it was also pointed out (I that in the cryptorchid child, FSH is mediator in the gonadal secretion of testosterone

Finally we can emphasize that in the adu diabetic alterations in the sexual function a found much more frequently in the first year the disease (70%) than in the subsequent yea (45%) (16) This appears to be due to the di ficult metabolic control before and after th diagnosis for a certain period of time and a pears to be at least partly supported by th negative correlation between LH and bloo glucose level (6) in the diabetic patient

If we consider that a good diabetic control difficult to obtain in childhood and that som of our patients received insulin for only a sho time before this investigation we have anoth possible explanation of our results

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SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS IN NORWEGIAN ADOLESCENT SCHOOL CHILDREN

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ABSTRACT Askevold R Høstmark A T Vellar O D von Kræmer Bryn M and Glattre E (Institute of Hygiene University of Oslo Oslo Norway) Serum cholesterol and triglyceride levels in Norwegian adolescent school children Acta Paediatr Scand 67 157 1978 —The frequency distribution of serum cholesterol and triglycerides in 172 boys and 232 girls 13–16 years from four elementary schools in Oslo has been determined The cholesterol values were significantly higher for girls 15–16 years than for boys of the same age group In the case of triglycerides boys 15–16 years had significantly higher values than boys 13–14 years Otherwise no statistically significant differences with regard to sex and age were observed The 85th percentiles have been suggested as appropriate upper normal limits In all groups the 85th percentile for plasma cholesterol was slightly below 6 mmol/l The corresponding plasma triglyceride value was below 2 mmol/l

KEY WORDS Serum cholesterol serum triglycerides school children

It is widely accepted that high levels of blood lipids are associated with increased risk in the development of cardiovascular diseases Clinical symptoms of atherosclerosis are late manifestations of the disease Early detection of factors of increased risk such as high blood lipid levels might improve the prevention of cardiovascular diseases

As shown by several workers the level of blood lipids vary with age and sex (1 3 4 6 8–10 14) There are also considerable variations between different populations (2 6 12) This means that in order to evaluate whether a given serum lipid concentration is normal or pathological it is of importance to estimate age sex and population specific upper limits for the blood lipids

Few studies on adolescent blood lipid values in Scandinavia have been published (4 7 15) In the present paper the distribution of serum cholesterol and triglycerides in a group of Norwegian school children is presented together with suggested upper normal limits for these blood lipids

MATERIALS AND METHODS

The subjects participating in this study were healthy adolescent school children in four elementary schools in Oslo Their sex weight and height are given in Table 1 Menarche was reported in 55% of girls aged 13–14 years and in 96% of girls aged 15–16 years

During 1974–75 blood samples were taken by puncture of an antecubital vein No instruction about fasting was given

Serum cholesterol was determined enzymatically according to Röschlau et al (11) using kits of Boehringer Serum triglycerides were determined according to Eggstein & Kreutz (5) using kit reagents (Boehringer) of glycero-kinase pyruvate kinase and lactic dehydrogenase The concentration of triglycerides was estimated from total glycerol values No correction was made for free glycerol which amounts to approximately 0 12 mmol/l The Student's *t* test was used to test the significance of differences between mean values

Table I *Sex age number of subjects body weight and height for the different groups of the material*

		Body weight (kg)			Height (cm)		
Age (y)	*n*	Range	M	S D	Range	M	S D
Girls							
13–14	106	30 8–79 3	50 7	8 8	142–178	161 6	6 6
15–16	126	41 0–81 0	56 4	7 5	151–180	166 3	5 6
Boys							
13–14	89	32 0–78 9	49 2	8 4	140–183	161 0	8 8
15–16	83	43 3–86 0	53 4	10 9	149–191	174 0	8 1

RESULTS

In girls and boys of both age groups an unimodal distribution for serum cholesterol was observed (Fig 1) The distribution of serum triglycerides was also unimodal but with a positive skewness in girls and boys of both age groups (Fig 2) In each sex, no statistically significant difference in mean serum cholesterol concentration between the age groups 13–14 years and 15–16 years was found (Table 2) However 15–16 years old girls had a significantly higher mean serum cholesterol level than boys of the same age group (Table 2) No statistically significant sex differences in mean serum triglyceride concentration were observed but 15–16 years old boys had a significantly higher serum triglyceride level than boys of the younger group

Different estimates of the upper limit of the normal range for serum cholesterol and triglycerides in both sexes and in age groups 13–14 years and 15–16 years are presented in Table 2 For reasons indicated below the 85th percentile is considered to be the most accurate estimate With regard to serum cholesterol the 85th percentile equals 5 22 mmol/l for boys 13–14 years old and 5 17 mmol/l for boys 15–16 years old The 85th percentile for girls equals 5 35 mmol/l in age group 13–14 years and 5 77 mmol/l in age group 15–16 years

With regard to serum triglycerides the 85th percentile equals 1 62 mmol/l and 1 87 mmol/l for boys 13–14 and 15–16 years respectively For girls the corresponding values are 1 77 mmol/l and 1 65 mmol/l

DISCUSSION

Estimation of the normal range of a variable by trimming the tails of the distribution of its values in a proper sample of healthy individuals is based on generally accepted principles

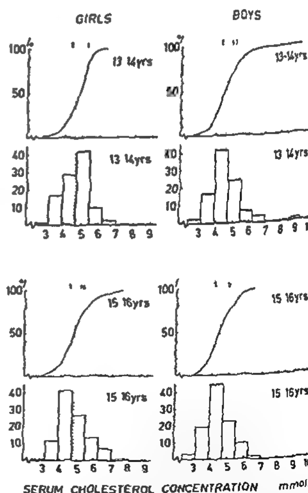


Fig 1 Frequency and cumulative frequency distribution of serum cholesterol concentration in girls and boys Upper panel 13–14 years Lower panel 15–16 years

Table 2 *Mean values and suggested upper "normal" limits for serum cholesterol and triglycerides in adolescent school children*

	Girls					Boys				
			Upper "normal" limits					Upper "normal" limits		
	n	Mean ±S E	Mean +2 S D	85th percentile	95th percentile	*n*	Mean ±S E	Mean +2 S D	85th percentile	95th percentile
Cholesterol (mmol/l)										
13–14 years	10	4 63±0 09	6 08	5 35	5 66	83	4 5[illegible]±0 08	6 38	5 22	5 95
15–16 years	119	4 76±0 06	6 41	5 77	6 33	76	4 40±0 10	5 79	5 17	5 51
Triglycerides (mmol/l)										
13–14 years	101	1 [illegible]6±0 05	2 26	1 77	2 13	81	1 15±0 06	[illegible] 18	1 6	[illegible] 13
15–16 years	117	1 [illegible]0±0 05	2 18	1 65	2 18	73	1 3[illegible]±0 06	2 39	1 86	2 33

p<0 005 vs boys of the same age group
p<0 03 vs boys 15–16 years old

Any set of criteria of good health will if not being too restrictive entail the possibility of including non healthy persons in the sample To the extent these persons represent extreme values of the given variable one circumvents the contamination problem by cutting off upper parts of the tails of the distribution On account of the skewness of some of the *present* distributions estimates of "normal" upper limits based on the median will be superior to estimates based on the mean

Since no instruction was given as to fasting a greater rather than a smaller part of the upper tail of the cholesterol and triglyceride distributions might be subtracted Further more lipid values in industrialized countries are considered to be elevated We accordingly suggest the 85th percentiles to be reasonable estimates of the "normal" upper limits These limits seem to be in accordance with current clinical practice

Direct comparison between lipid levels obtained in various investigations is difficult to perform due to different analytical methods The enzymatic method used in the present study for cholesterol determination yields e g somewhat lower values than the commonly used Liebermann Burchard method (11)

Comparisons between different studies regarding serum lipid variations with sex and age

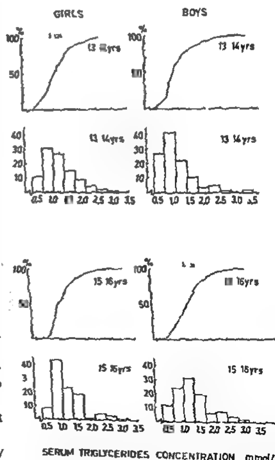


Fig 2 Frequency and cumulative frequency distribution of serum triglycerides concentration in girls and boys *Upper panel* 13–14 years *Lower panel* 15–16 years

may however be done irrespective of methods used Our finding that the serum level of triglycerides in boys increased with age is in accordance with results obtained in previous studies (4 8) The observation that boys and girls 15–16 years had similar triglyceride levels is in accordance with results reported by Berenson et al (1)

However, our finding that the mean serum cholesterol concentration did not change significantly from the age 13–14 to 15–16 years in both sexes is in contrast to the observation made by these authors who reported that serum cholesterol like triglycerides increased in both sexes Srinivasan et al (12) observed on the contrary a decrease in serum cholesterol for girls and possibly for boys from the age 13–14 years to the age of 15–16 years Furthermore the observation of the present study that serum cholesterol of girls 15–16 years was significantly higher than for boys of the same age is not in accordance with previous studies by Lauer et al (10) and by Starr (13) who reported no sex differences in school children These discrepancies might reflect different geographical, ethnic and possibly other factors and illustrate the need for population specific reference values

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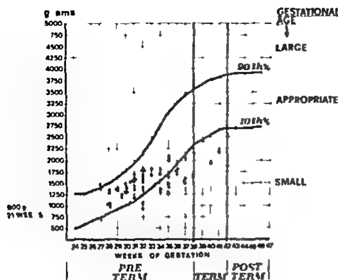


Fig 1 Classification of newborns according to birth weight and gestational age (Lubchenko) Points represent newborns which survived during the study period crosses represent deceased infants

was used developed necrotizing enterocolitis Qualitative changes in the bacterial microflora in infants fed by transpyloric tube were demonstrated by Challacombe (3 4) A case of duodenal perforation was encountered by Sun (20) All the authors who found such complications used polyvinyl tubes which remained in the bowel several days (whole weeks in some cases) without being changed and did not mention osmolality data of the feeding

In 1974 Ekert (8) simplified the technique by introducing the polyvinyl tube directly into the duodenum without a weighted tip or a double tube The tube was changed every fourth day No serious complications were found in more than 160 infants treated in 1974 and 1975 (9) Van Caillie and Powell (21) confirmed the practicality and safety of nasoduodenal feeding in 12 very low birth weight infants by comparison with the same number of infants fed by continuous nasogastric feeding

The aim of this investigation was to check on the basis of a clinical trial carried out on a vast number of high risk infants the validity of the nasoduodenal alimentation The trial was planned in order to 1) assess if it really is a good feeding method 2) study the effect on the ponderal increment and its caloric cost 3) examine if complications could be expected and finally 4) study its influence on the various type of neonatal risk

PATIENTS AND METHODS

Study population

Out of 673 babies admitted to our Neonatal Intensive Care Unit (N I C U) from 16th February 1975 to 6th June 1976 and coming either from our delivery room (596 newborns) or from other hospitals (77 newborns) 100 were fed by nasoduodenal tube (87 coming from inside 13 from outside) They were classified into four cohorts on the basis of birth weight in 500 g divisions

Cohort 1 Eight of 17 infants with a birth weight of 1000 g or less (47 %)

Cohort 2 Forty-one of 61 infants with a birth weight o 1001–1500 g (67 2 %)

Cohort 3 Thirty eight of 98 infants with a birth weight o 1501–2000 g (38 7 %)

Cohort 4 Thirteen of 497 infants with a birth weight o more than 2000 g (2 6 %)

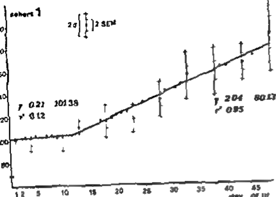
The first two cohorts included all infants admitted apart from those who had serious haemorrhagic problem or very severe respiratory distress The cohorts 3 and 4 in cluded only those infants who had evidence on admissio of fetal malnutrition dystrophy difficulty in sucking o repeated vomiting during nasogastric or oral alimentatio Most of the infants were pre term (90 out of 100) and majority (63 out of 100) appropriate for gestational a (Fig 1)

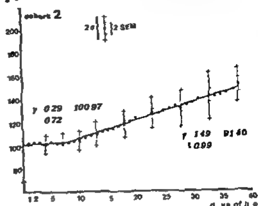
The time of start of nasoduodenal feeding depended o the moment of admission of newborns in our N I C U which varied because some of them came from oth hospitals when the resident physician decided need fo transfer Infants started nasoduodenal feeding in the fir 2 days of life with the exception of 3 neonates in cohort who developed severe vomiting during oral alimentati and started nasoduodenal later between 8 and 14 days life

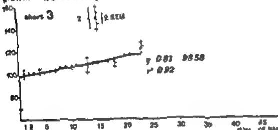
Duration of treatment depended on the state of the new born Treatment was stopped when a good weight incr ment was obtained and it was felt that the infant cou manage without it or when the pathological conditio requiring nasoduodenal feeding were resolved

Administration technique

The 100 infants were treated according to the techniq described by Ekert (8) In brief the infant was plac supine or on the right side and after stomach aspiratio a polyvinyl chloride tube (Pharmaseal K 32 FG 5) was i serted via the nose When the tube aspirate changed fro strongly acid (pH 3 5 to 4 5) to less acid values (pH 6 to 6 5) the tube tip was deemed to have passed t pylorus The pH of the aspirate was checked three tim in 24 hours to ascertain correct placing of the polyvin tube in the duodenum Following this technique it w not necessary to execute a series of roentgenograms control the tube tip position every time it was introduce The tube was changed every fourth day







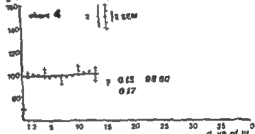


Fig 2 Growth as per cent of birth weight versus days of life The points are the values of the means to some of which the dispersion (σ) and S E M are associated The straight lines are the fitting functions whose equations and correlation coefficients are reported near them The equations ( ) are those used for the *t* test

Alimentation formula and infusion rate

Breast milk if available and/or a formula (Humana O) containing 2 3% protein with a whey protein/casein ratio of 60 40 3 3% fat with essential fatty acids forming 15% of total lipid 8 6% carbohydrate osmolality 387 mOsm/kg and 75 kcal/100 ml was administered at a constant and continuous rate by an infusion pump (5003 Infors AG) The infusion rate during the first day was 60–70 ml/kg increasing daily thereafter by 10–15 ml/kg until a caloric intake of 140–150 kcal/kg per day was reached The fluid intake then varied between 209 and 2?4 ml/kg per day when this formula was used

All infants in this study who needed parenteral infusions to correct biochemical imbalances such as electrolytes or bicarbonate received these in 5% glucose until the imbalances were corrected as we do routinely in these cases No other parenteral sources of calories were administered

RESULTS

The effect of nasoduodenal feeding has been measured in several ways but particular consideration has been given to (*a*) weight gain (*b*) caloric cost (*c*) mortality rate observed in the Unit before and after the introduction of this nutritional regime and (*d*) complications

Weight gain

The weight gain of the newborns of each cohort was evaluated by daily recordings For each newborn under test the mean of percentage weight variation referred to birth weight was calculated for every day The results were transferred on graphs (Fig 2) From all the points of each cohort some were chosen and studied to define the dispersion from the weight variation mean (σ) and the standard error of the mean (S E M) and these values were added to the graphs (Fig 2) Obviously as the days went by the dispersion

Table 1 *Weight gain for each cohort expressed as g/day and referred to birth weight gestational age time of start and duration of nasoduodenal feeding with dispersion (σ) and S E M*

	Weight gain (g/day)			Birth weight (g)			Gestational age (weeks)			Time of start (hours)			Duration (days)		
Cohort	Mean	σ	S E M	Mean	σ	S E M	Mean	σ	S E M	Mean	σ	S E M	Mean	σ	S E M
1	17 2	3 8	1 5	865	118 9	48 5	27	3	1	11	7	3	53	9	4
2	17 4	6 3	1 0	1 309	142 4	23 4	31	3	1	18	43	8	29	9	1
3	13 7	11 7	1 9	1 720	124 8	20 5	34	3	1	24	41	7	15	9	1
4	20 8	24 2	7 0	2 260	234 8	67 8	37	3	1	80	117	34	8	5	1

and S E M tended to increase since the number of newborns fed by the nasoduodenal method decreased An estimation of the function weight variation versus life days can be obtained from each graph A linear regression method was adopted to fit these functions (Fig 2) The t test was used to test the significance of the difference between the slopes and we can affirm that there is a probability of less than 5% that the values of the slopes have been obtained casually

Table 1 shows for each cohort the mean weight gain expressed as g/day and referred to birth weight gestational age starting time

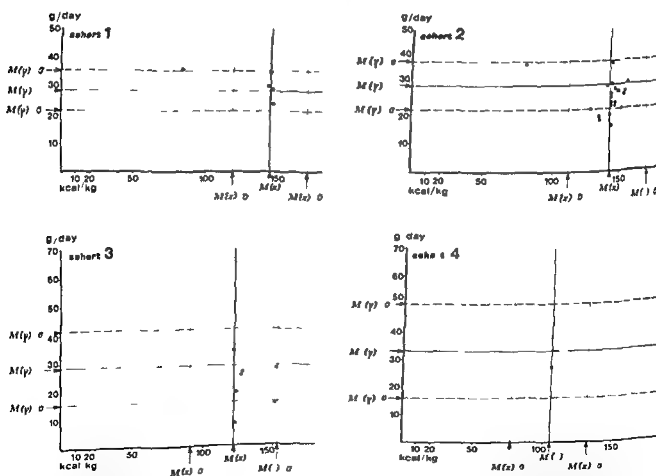


Fig 3 Scatter diagrams points represent newborns x axis represents calories assumed pro kilo and y axis the daily growth in the period of the best weight gain for each newborn Pondered mean values of calories assumed $M(x)$ and of daily growth $M(y)$ are represented by orthogonal lines the dotted lines represent the dispersion from the mean

Table 2 *Pondered means of caloric intake (M(x)) and of weight gain (M(y)) with dispersion (σ) and S E M and weight gain per unit of caloric intake for each cohort*

The data have been calculated on the period of the best weight gain

Cohort	Caloric intake (kcal/kg)			Weight gain (g/day)			Weight gain per unit of caloric intake (g/100 kcal)
	M(x)	*σ*	S E M	*M(y)*	*σ*	S E M	
1	145 0	‑6 0	9 2	?8 7	6 9	2 5	2? 9
?	14? 9	31 ?	4 9	30 5	8 6	1 3	16 3
3	1?? ?	31 1	5 0	?8 8	13 1	? 1	13 7
4	104 6	?7 3	7 6	33 1	17 1	4 3	14 0

and duration of nasoduodenal feeding with dispersion and S E M

Caloric cost

The best ponderal increment period was considered for each of 100 newborns Caloric administration and daily growth represented two series of data whose statistical distribution was *a priori* unknown However it was possible to construct a scatter diagram (Fig 3) showing the distribution of the cases in each cohort Cohorts 1 and 2 presented a high concentration of cases in a well defined area while the cases of the other two cohorts were widely dispersed

A possible connection of the caloric intake with daily growth was submitted to statistical analysis This demonstrated the existence of a connection although not very marked It was therefore decided to estimate the regression of the function g/day=F (kcal/kg) and of the inverse function kcal/kg=F (g/day) For the first three cohorts the two functions g/day versus kcal/kg and kcal/kg versus g/day were quite constant round their mean values M (y) and M (x) These values are reported in Table 2 with weight gain per unit of caloric intake

Mortality rate

The mortality rate in our Unit was studied before and after the introduction of the new feeding technique (Fig 4) The graph shows that the mortality rate fell considerably in 1975 the year nasoduodenal feeding was introduced The decrease was particularly evident in neonates with a birth weight of 1 000 g or less the rate for this group being 100% in 1974 76 9% in 1975 and finally 73 3% in 1976 Infants with a birth weight between 1 001 and 1 500 g had a mortality rate of 60 9% in 1974 33 3% in 1975 and 46 1% in 1976 In the newborns with a birth weight over 1 500 g the mortality rate was not statistically influenced by the new feeding technique As a consequence of the considerable decrease in mortality of the newborns with a birth weight below 1 500 g the overall mortality rate at our Unit fell considerably As a matter of fact the mortality rate in the Unit was showing a slight fall in the last 3 years before the introduction of the

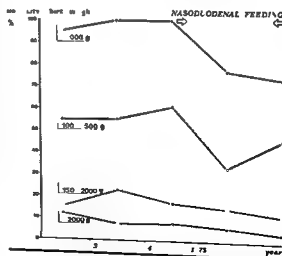


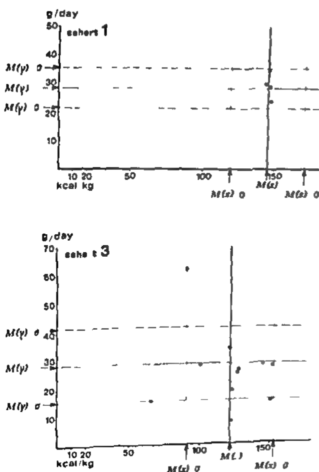
Fig 4 Mortality rate in our N I C U before and after the introduction of nasoduodenal feeding The newborns have been divided on the basis of birth weight in 500 g divisions

Table 1 *Weight gain for each cohort expressed as g/day and referred to birth weight gestational age time of start and duration of nasoduodenal feeding with dispersion (σ) and S E M*

Cohort	Weight gain (g/day)			Birth weight (g)			Gestational age (weeks)			Time of start (hours)			Duration (days)		
	Mean	σ	S E M	Mean	σ	S E M	Mean	σ	S E M	Mean	σ	S E M	Mean	σ	S E M
1	17 2	3 8	1 5	865	118 9	48 5	27	3	1	11	7	3	53	9	4
2	17 4	6 3	1 0	1 308	142 4	23 4	31	3	1	18	43	8	29	9	1
3	13 7	11 7	1 9	1 720	124 8	20 5	34	3	1	24	41	7	15	9	1
4	20 8	24 2	7 0	2 260	234 8	67 8	37	3	1	80	117	34	8	5	1

and S E M tended to increase since the number of newborns fed by the nasoduodenal method decreased An estimation of the function weight variation versus life days can be obtained from each graph A linear regression method was adopted to fit these functions (Fig 2) The *t* test was used to test the significance of the difference between the slopes and we can affirm that there is a probability of less than 5% that the values of the slopes have been obtained casually

Table 1 shows for each cohort the mean weight gain expressed as g/day and referred to birth weight gestational age starting time



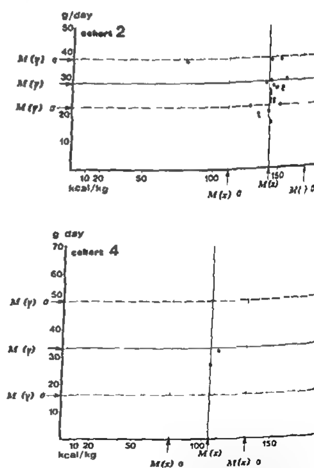


Fig 3 Scatter diagrams points represent newborns *x* axis represents calories assumed pro kilo and *y* axis the daily growth in the period of the best weight gain for each newborn Pondered mean values of calories assumed *M* (*x*) and of daily growth *M* (*y*) are represented by orthogonal lines the dotted lines represent the dispersion from the mean

23 g/100 kcal in cohort 1 to 14 g/100 kcal in cohorts 3 and 4 (Table 2) showing a steady decrease in larger infants Now this means that smaller infants gain weight more rapidly and consume more calories than do larger infants

Mortality rate

After the introduction of nasoduodenal feeding the mortality rate in our Unit fell considerably particularly in newborns with a birth weight less than 1500 g For newborns with a birth weight more than 1500 g it was not significantly influenced because the number of infants treated was low and feeding was not the predominant factor in the assistance

The decrease of mortality in the newborns with a birth weight less than 1500 g was so high as to influence the total mortality in our Unit (from 18% in 1974 to 11 4% in 1975)

Although it is not correct to compare the results of mortality obtained in different time periods nevertheless since no other aspect of neonatal care in the Unit changed during the study period we are reasonably inclined to believe that this mortality fall has been caused by the introduction of the new feeding technique

The slight and constant fall shown in 1973 and 1974 was very likely only due to an improvement in general management particularly in respiratory assistance The slight increase of mortality observed in 1976 in the newborns between 1001 g and 1500 g is according to our interpretation due to a higher percentage of infants coming from outside 36% in 1976 against 21% in 1975 and 24% in 1974

Complications

The complications attributable to the transpyloric feeding and reported in the literature namely diarrhea (4 13) necrotizing enterocolitis (2 6 13) intestinal perforations (2 6 15 20) and impaired assimilation of food (19) were not found On this subject it is interesting to observe that in the literature the osmolality of the transpyloric feed has been nearly always neglected with some exceptions (5 18) who underlined the importance of this factor (7 10) No real details on the osmolality of the formula used are given in any of the cases concerning inflammatory complications (necrotizing enterocolitis) (2 6 13) A formula of less than 400 mOsm/kg has always been supplied to our treated newborns as this is considered to fall within a reasonable caution limit It has been one of our main concerns to stay within this limit

The intestinal perforations reported in the literature can be prevented if a simple precaution is observed to change regularly the transpyloric polyvinyl chloride tube which should never be left *in situ* more than 3 consecutive days In fact according to the investigations of Henderson & Alden (14) and in our own experience the consistency of the polyvinyl tube gradually modifies from soft to stiff becoming potentially dangerous from the 4th day on In very small newborns polyvinyl tubes are to be preferred to silicone ones because the extreme pliability and softness of the latter very often hinder passage of the tube direct through the pylorus and furthermore the smallest silicone tubes are so limp and soft that they usually collapse under attempts at suction aspiration

Local vascular changes and high osmolality together with a stiff tube can cause a necrotizing enterocolitis or perforation These complications can be avoided and prevented if the two above mentioned precautions are observed iso-osmolar transpyloric formula and frequent changes of the polyvinyl chloride tube

Our conclusion is that this form of feeding is suitable for all high risk infants and particularly those with a birth weight of 1500 g or less

ACKNOWLEDGEMENTS

The authors wish to thank Professor R D G Milner Head of the Department of Paediatrics Sheffield University for his expert supervision and Doctor N Raiha Departments of Obstetrics and Gynaecology and Paediatrics

new feeding technique (23 6% in 1972 19 8% in 1973 18% in 1974), while in 1975 the year nasoduodenal feeding was introduced it fell considerably to a value of 11 4% which remained practically constant in 1976 (11 1%) This assurance was submitted to statistical analysis The χ^2 test showed that there is less than 1% probability that this fall was not due to some change in assistance techniques

Complications

Nine infants out of 100 died 2 out of 8 in cohort 1 5 out of 41 in cohort 2 one each in cohorts 3 and 4 The autopsies revealed that in no case was death related to the feeding technique No evidence of inflammatory changes in the intestinal tract could be found histologically Causes of death were hyaline membrane disease in 4 cases subarachnoid haemorrhage neonatal pneumonia sepsis and unstable lung in one case One dead infant weighed only 900 g and had a gestational age of 21 weeks

In only one case nasoduodenal feeding was stopped after 9 days because the infant with a birth weight of 1150 g contracted rhinitis probably caused by the tube

DISCUSSION

The duodenum rather than the jejunum was chosen for this clinical trial the reason being that the former serves an important function in digestion and that the by pass of this intestinal tract could present some impairment in digestion and adsorption processes

Weight gain

No physiological weight loss in each cohort was observed (Fig 2) This was probably because nasoduodenal feeding allowed an adequate intake of essential nutrients and calories immediately after birth Therefore all the infants were maintained in an anabolic state despite their very low birth weight and/or illness Moreover the smallest newborns had a greater growth rate If the growth rate of cohort 1 is referred to 100% the newborns of cohort 2 had a growth rate of 73% of cohort 3 of 39% and of cohort 4 of 7% Considering the mean weight gain for each cohort in the period of the best weight gain (Table 2) it can also be inferred that the premature infants have a growth rate potentially greater than mature infants This confirms the opinion of Babson (1) and Fomon (11) who compared the growth rates of premature infants with those of the fetuses

Caloric cost

An important fact stands out from statistical analysis In the first three cohorts the two functions g/day vs kcal/kg and kcal/kg vs g/day result quite constant round their mean values Moreover always for the first three cohorts the weight gain per day is fairly constant (round 29 g) This probably means that the weight gain is independent within certain limits from caloric administration and birth weight

Instead for cohort 4 the two functions g/day vs kcal/kg and kcal/kg vs g/day are not constant and the caloric performance lies in the interval from 15 to 40 g/day referred to an interval from 90 to 130 kcal/kg This is presumably due to the fact that the feeding was not the predominant factor in the assistance of the infants

The mean caloric intake is greater in smaller infants than in larger ones (Table 2) This probably reflects the greater caloric needs for growth of the more rapidly growing smaller infants (11) The low birth weight infants have an increased caloric requirement per unit of body weight whether born prematurely or are small for gestational age

As far as weight increase is concerned the values reported in Table 2 show the pondered means of weight gain referred exclusively to the period of highest weight gain and should not be considered as the means of general growth

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LEFT SIDE PREFERENCE IN HOLDING AND CARRYING NEWBORN INFANTS

I *Mothers Holding and Carrying during the First Week of Life*

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ABSTRACT de Chateau P Holmberg H and Winberg J (Departments of Paediatrics and Obstetrics University of Umeå Umeå and the Department of Paediatrics Karolinska Institute Karolinska Hospital Stockholm Sweden) Left side preference in holding and carrying newborn infants I Mothers holding and carrying during the first week of life Acta Paediatr Scand 67 169 1978.—Eighty to ninety per cent of primiparous and multiparous women held their newborn infants to the left of the body midline Left side preference was independent of handedness and was typical also of non pregnant female—but not male—students and may therefore be a genetically determined human female behaviour Right holding occurring in 10 to 20% of non-separated women was observed in 30 to 40% when mother and infant had been separated for about 24 hours This observation may be of general interest suggesting that perinatal maternal anxiety and uncertainty can alter a pre-existing behaviour The right holding non separated mothers differed from left holding ones in a number of ways they held their babies with less body contact they perceived a delay in accepting the foetus or newborn as their own and—when checked 3 years after delivery—they had had a more frequent contact with the Child Health Center during this time Right holding may in some mothers be an early sign of a disturbed mother–infant relationship It may indicate either an insensitivity of the mother to the signals of the infant or that the infants signals are in appropriate Carrying differed somewhat from holding In addition to right and left preference a third modality— in hands —was observed Immediate post partum naked skin to-skin and suckling contact between mother and infant eliminated the carrying in hands behaviour This observation adds to others showing that experiences during the immediate postnatal period may mould the maternal behaviour pattern

KEY WORDS Maternal behaviour holding carrying early contact neonatal period

A left side preference for holding babies under 1 year of age was observed independent of handedness in 80% of mothers visiting well baby clinics (12 16) When mothers and infants had been separated immediately after birth the incidence of left holding was lowered (14) In the present study it was examined whether this preference already is present during the first days following delivery Carrying behaviour was also studied and an attempt was made to identify factors influencing these behaviours Left and right holding mothers and infants were followed for 3 years after delivery

MATERIAL

1 *Holding and carrying behaviour studied in 268 women 3–6 days after delivery*

Non separated group 228 mothers of healthy fullterm babies given routine post delivery care in a modern obstetric hospital as described elsewhere (7)

Separated group 40 mothers separated from their infants for at least 24 hours after delivery because of mal

Helsinki University Central Hospital for his valuable advice

They are also indebted to their Department staff and in particular the nurses for the help in collecting data and Mr S Norman for assistance and translation

Doctor C Sartori has kindly collaborated in the statistical analyses

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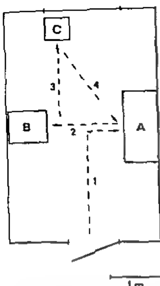


Fig 2 The room in which the observations were made *A*=nursing table *B*=crib *C*=chair Arrows 1–4 indicate the mothers position and way through the observation room ?=recording how the mother carried her infant (left right in hands) At *C* (chair) record was made of the side of the body on which the mother was holding her infant and whether she held the infant with close body contact or not

?) The mother was asked to take her infant from the table (A) and carry him to the crib (B) and to put him down Note was taken of whether the mother was carrying the infant in her hands or in her arms and in the latter instance on which side of her body The mother was then asked to pick up her infant again walk over to the chair (C) and sit down with the infant When the mother was sitting on the chair (C) (Fig) we observed on which side of her body she held her infant and whether she held her infant close against or away from her body The observer was standing close by in front of the mother

The students

The medical and nursing students were submitted to the same observations as the mothers see above

Primiparae with ext a contact and with routine care We observed how primiparae with (n=?) and without (n=?0) extra contact immediately after delivery carried their infants from the crib to the mother's bed before breast feeding Observations were made approximately 36 hours after delivery Out of 2 mothers with "extra contact one asked the nurse aid to fetch the infant from the crib and bring him to her bed Corresponding frequency among 0 mothers with routine care was five No observations could therefore be made of carrying behaviour in these six mothers

The follow up study

The follow-up study of the non-separated right holding mothers and their controls was made by means of a questionnaire 3 years after the original observation at the maternity ward The items covered included pregnancy and delivery socio-economic circumstances relationship to family and husband and child development The questionnaire contained yes or no questions open ended questions and questions to be answered on a scale from 1 to 5 Furthermore data were collected on mother s earlier and present obstetrical history from the records of antenatal clinics and hospitals Data concerning the children s perinatal period and the first 3 year period were collected from hospital and Child Health Care records The questionnaires and records were analyzed without knowledge of whether mothers had been right or left holding in the post-delivery period

During the observations in the maternity ward the mothers had not been aware of what was being recorded in their behaviour This is especially necessary when only a few items are registered The presence of an observer may be disconcerting (9) but mothers during their stay in the maternity ward are used to contact with the hospital staff e g lectures on infant care and did not show any signs of discomfort during the observation The mothers who held and carried their infants to the right of their body did not know that they belonged to a minority which could have influenced them during the follow up period The staff at the Child Health Centres was not informed about our particular interest in the families included in the study The mothers were not studied directly during pregnancy in a currently finished investigation however interviews were made one month prior to delivery (4)

Indirect procedures for studying mother–infant relationship such as interviews and questionnaires have limitations (11) Mothers may have a limited memory of things that have happened in the past and retrospective reports should therefore be avoided Mothers may moreover give a distorted report of their relationship with their children being influenced by what they believe is culturally expected mother–infant behaviour As mothers often have difficulty in recalling data exactly from the recent and more distant past both on herself and her child other ways of collecting data have been used By making direct observations of behaviour and using objective data from existing records made by third persons not aware of our interest in particular facts circumstances or measurements we feel that some of the disadvantages of interviews and questionnaires have possibly been compensated for (3) However even with the help of several different techniques of investigation it is often difficult to explain a correlation between a certain behaviour and data from the present or the past

RESULTS

1 (*a*) *Holding and carrying in right handed mothers*

Between 80–90% of primiparous and multiparous women *held* the infant to the left of the body midline (Table 2) Neonatal separation was associated with a doubling of right hold

Table 1 *Material examined for holding and carrying*

	Mean age (y)	Earlier contact with infants or small children (%)	Right handed (%)	Sex of infant F/M
Non separated group (n=228)				
Primiparae (*n*=133)	25 2	45	94	65/68
Multiparae (*n*=95)	29 2	100	94	45/50
Separated group (n=40)				
Primiparae (*n*=21)	25 4	39	100	10/11
Multiparae (*n*=19)	29 0	100	95	8/11

formation (*n*=1 pes equino-varus) postnatal observation (*n*=31) due to mild forms of postnatal asphyxia jaundice or disturbed regulation of body temperature and moderate prematurity (*n*=8) None of the 40 infants was severely ill and all were healthy at discharge Clinical data of the mothers and babies are given in Table 1

2 *Holding and carrying behaviour in students*

Twenty eight female and 21 male right handed students who had no children of their own but who all had professional contact with both infants and children

3 *Carrying behaviour in mothers with immediate post delivery skin to-skin contact*

The carrying behaviour was studied in 22 primiparae who had had 15–20 min immediate postnatal naked skin to-skin and suckling contact (extra contact) with their newborns Twenty primiparae with routine care served as controls (2)

4 *Follow up 3 years after delivery of non separated right handed mothers who during the neonatal period held their infants to the right*

Thirty five out of 37 non separated *right holding* mothers were compared to 35 non separated *left holding* mothers The groups were matched for maternal age parity and sex of infant

METHODS

Fig 1 illustrates different ways of holding and carrying the baby Definitions are as follows

Holding to the left (right) mother holding infant in her arms the infant s head pointing to the left (right) side of the mother s body

Carrying to the left (right) mother walking with infant in her arms infant s head pointing to the left (right) side of the mother s body

Carrying in the hands mother walking with infant in her hands not in her arms without body contact and regardless of to which side of the mother s body the infant s head is pointing

Body contact at least half of the infant s body in direct contact with mother s body while holding

The non separated and separated mothers

All observations were made between the 2nd and 8th day after delivery (mean non separated mothers 4 2 days S D ±1 3 and separated mothers 5 4 days S D ±1.3) by one observer in a room furnished as shown in Fig 2 Before entering this room all mothers were instructed by the experimenter who then followed them in The mother s own infant was placed on the table with his feet towards her so the mother was able to make her own choice as to the side on which she would pick up the infant The mother entered the room as indicated by the arrow 1 and her way through the room is shown by arrows 2–4 (Fig



Fig 1 (a b) Sitting holding to the left and to the right respectively (*c d*) carrying to the left and to the right (*e*) carrying in hands (*f*) holding away from the body (as opposed to *a* and *b*)

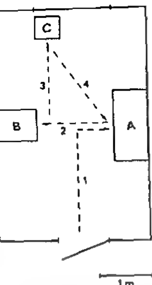


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Primiparae (n=21)	25.4	39	100	10/11
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formation (n=1 pes equino-varus) postnatal observation (n=31) due to mild forms of postnatal asphyxia jaundice or disturbed regulation of body temperature and moderate prematurity (n=8) None of the 40 infants was severely ill and all were healthy at discharge Clinical data of the mothers and babies are given in Table 1

2 Holding and carrying behaviour in students

Twenty eight female and 21 male right handed students who had no children of their own but who all had professional contact with both infants and children

3 Carrying behaviour in mothers with immediate post delivery skin to-skin contact

The carrying behaviour was studied in 22 primiparae who had had 15–20 min immediate postnatal naked skin to-skin and suckling contact (extra contact) with their newborns Twenty primiparae with routine care served as controls (2)

4 Follow up 3 years after delivery of non separated right handed mothers who during the neonatal period held their infants to the right

Thirty five out of 37 non separated *right holding* mothers were compared to 35 non separated *left holding* mothers The groups were matched for maternal age parity and sex of infant

METHODS

Fig 1 illustrates different ways of holding and carrying the baby Definitions are as follows

Holding to the left (right) mother holding infant in her arms the infant s head pointing to the left (right) side of the mother s body

Carrying to the left (right) mother walking with infant in her arms infant s head pointing to the left (right) side of the mother s body

Carrying in the hands mother walking with infant in her hands not in her arms without body contact and regardless of to which side of the mother s body the infant s head is pointing

Body contact at least half of the infant s body in direct contact with mother s body while holding

The non separated and separated mothers

All observations were made between the 2nd and 8th after delivery (mean non separated mothers 4.? d S D ±1 3 and separated mothers 5 4 days S D ±1.3 one observer in a room furnished as shown in Fig 2 fore entering this room all mothers were instructed by experimenter who then followed them in The mo own infant was placed on the table with his feet tow her so the mother was able to make her own choic to the side on which she would pick up the infant mother entered the room as indicated by the arrow 1 her way through the room is shown by arrows 2–4



Fig 1 (*a b*) Sitting holding to the left and to the ri respectively (*c d*) carrying to the left and to the rig (*e*) carrying in hands (*f*) holding away from the bo (as opposed to *a* and *b*)

Table 5 *Holding behaviour in right handed students*

	Holding to the left
Females (n=28)	26
Males (n=71)	13

$p < 0.05$

The extra contact group had a naked skin to-skin and suckling contact with their babies within the first hour of life The controls were cared for according to the routine (2) The setting of the observation did not allow a study of the holding behaviour (2) The distribution of carrying in the routine care group is in full consistence with the results in the larger material (Tables 2 and 6) In the extra contact group carrying in the hands had disappeared completely

4 Follow up of 70 non separated mothers and infants three years after delivery

Since such a small minority of the mothers to healthy fullterm infants held their babies to the right (Table 2) we decided to see whether these mothers differed from left holding ones as to their background factors Thirty five right holding mothers to the healthy fullterm infants were compared to a group of 35 left holding mothers No differences were found between the two groups with regard to the number of abortions complications during pregnancy and delivery medication during and mode of delivery and duration of labour Socio-economic background factors such as housing occupation education and composition of the family were comparable in both groups The infant s conditions as measured by Apgar score birth weight and neonatal adaptation were similar and in both groups all infants had been with their mothers in the maternity ward

Non separated mothers holding to the right reported in a higher proportion than those holding to the left that it had taken long time for them to relate to and accept their feelings towards their growing fetus or newborn infant (Table 7) Similarly non separated mothers holding to the right both primiparae and multiparae had more frequent contact with the Child Health Centre and got significantly more home visits from the district nurses during the follow up period (Table 7)

DISCUSSION

The present study has shown that some 80% of non separated mothers hold their fullterm healthy newborns to the left of the body mid line This behaviour seems to be independent of handedness The findings are similar to those of Salk (14) and Weiland (16) who observed mothers of older infants The present study could however not corroborate Salk s finding (14) that early contact with a previous child influenced holding of the next infant

The preference for holding to the left does not seem to be elicited by pregnancy or delivery It was already observed in female students and seems to develop during childhood in females (1) It may thus be a genetically determined human female behaviour It is also said to occur in other primates (13) The observation that separation of mother and child during the neonatal period abolished side preference may be of a general interest suggesting that perinatal maternal anxiety and uncertainty can alter a pre existing behaviour The left side preference seems to be more typical for females since this was not observed in

Table 6 *Influence of immediate post delivery naked skin to-skin and suckling contact (extra contact) on primiparae s carrying behaviour*

	Extra contact care (n=21)	Routine care (n=15)
Carrying to the left	18	7
Carrying in the hands	0	5
Carrying to the right	3	3

$p < 0.05$

Table 2 *Holding (sitting on chair C Fig 2) and carrying (moving along arrow 2 Fig 2) in 215 non separated and 39 separated right handed mothers*

	Primiparae		Multiparae	
	Non separated ($n=125$) (%)	Separated ($n=21$) (%)	Non separated ($n=90$) (%)	Separated ($n=18$) (%)
Hold left	80	62	86	61
Hold right	20	38	14	39
Carry left	54	43	73	66
Carry in hands	31	39	13	17
Carry right	15	18	14	17

* $p<0.05$

ing Holding to the right was associated with less body contact than holding to the left both in non separated and separated pairs (Table 3)

Carrying differed from *holding* in a number of ways in addition to right and left preference a third modality in hands (see definitions and Fig 1) was observed Left side preference was not as marked and parity more than separation seemed to influence this behaviour (Table 2) Side preference was a stable phenomenon only 3% among the mothers holding to the left and 6% among those holding to the right changing side from holding to carrying The sex of the infant and the actual time of observation (day after delivery) did not influence the holding or carrying behaviour in either separated or non separated mothers

1 (*b*) *Holding in left handed mothers (non separated only)*

Ten out of 13 held their babies to the left In Table 4 our comparatively small material is added to the materials of Salk (13 14) and of Weiland (16) which are also comparatively small The trend is similar in all the materials handedness has little or no influence on the holding behaviour

Table 3 *Body contact while holding infant sitting in chair C Fig 2 (right handed mothers only)*

	With body contact	
	Non separated ($n=215$) (%)	Separated ($n=39$) (%)
Holding to the left	95	75
Holding to the right	82	53

All separated vs all non separated pairs $p<0.001$

2 *Holding and carrying behaviour in female and male right handed students*

The results are given in Table 5 In this small series the female students *held* the infant significantly more often to the left of the body midline than their male colleagues did The females *carried* the infants similarly to the non separated mothers while the males carried somewhat more to the right and in the hands

3 *Carrying behaviour in mothers with immediate post partum naked skin to skin contact*

Approximately 36 hours after delivery two groups of primiparous women were studied

Table 4 *Side of body against which the left handed non separated mothers held their infants*

	Hold left
Present study ($n=13$)	10
Salk ($n=32$)	25
Weiland ($n=5$)	4
Total ($n=50$)	39

Table 5 *Holding behaviour in right handed students*

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Females (*n*= 8)	76
Males (*n*=71)	13

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Table 7 *Follow up of 35 leftholding and 35 rightholding non separated mothers three years after delivery*

Primi right (Primi left) = primiparae holding infant to the right (left) during the neonatal week Multi right (Multi left) multiparae holding infant to the right (left) during the neonatal week

	Primileft (n=20)	Primiright (n=20)	Multileft (n=15)	Multiright (n=15)
Acceptance of foetus (infant) as own (Approximate mean values)	At delivery	Later	During first part of pregnancy	During second part of pregnancy
Mean numbers of home visits by the Child Health Centre nurse	2 2*	4 2*	2 1	3 8
Mean numbers of all contacts between mother and Child Health Centre	15 4	17 5	12 7	14 9

* $p<0.05$

male students nor in boys (1) In a systemic study of art infants and children were in about 80% of cases held to the left side in females (5) No such side preference was found in male holders (5) Fathers are presently being observed

The right holding non separated mothers were found to hold their babies with less body contact than the left holding Holding with little body contact during the neonatal period has earlier been found to be associated with a disturbance in mother–infant relations 12 months after delivery (6 8) Holding to the right may therefore be another early sign of a disturbed mother–infant relationship This possibility was further supported by our follow up 3 years after delivery mothers with healthy infants holding to the right had more contact with the Child Health Centre possibly indicating greater need for help among them than among those holding to the left The right holding mothers also accepted the fetus or baby as theirs at a later time after conception than did mothers holding to the left which might suggest that the two categories already differed during gestation

The side preference in carrying infants does not seem to have been studied earlier Comparison of holding and carrying showed that the left side preference was a stable phenomenon It is also of interest that 15 min of naked mother–infant contact immediately after delivery completely eliminated the carrying in hands behaviour This observation adds to others showing how the immediate postnatal period may mould the maternal behaviour pattern (2 3 6 7 8)

In explaining the left side preference in holding infants Salk (13) discussed the influence of the maternal heartbeat as an imprinting stimulant with a soothing effect on the infant Weiland & Sperber (17) postulated that the preference for holding to the left primarily serves to relieve anxiety in the adult carrier It is also possible that the infant plays a key role in the forming of maternal behaviour patterns Miranda (10) showed that when newborn infants were presented with two identical pictures the infants more often looked at the one on the right than the one on the left Furthermore Turkewitz et al (15) have shown that infants more often turn their head to the right than to the left when stimulated on the cheek This may be a species specific adaptation to maternal holding on the left i e when looking to the right the baby can see his mother But this infant behaviour could also be a signal to the mother favouring holding to the left Deviations from the norm in holding might then indicate either an insensitivity on the part of the mother to the infant s signals or that these signals are inappropriate Such explanation

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ACKNOWLEDGEMENT

This study was supported by grants from the Åhlén Foundation and University of Umeå Statistical analyses were carried out by Lennart Nilsson and Anders Nylén Institution of Mathematics and Statistics University of Umeå

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BRAIN TYPICAL CREATINEKINASE IN THE SERUM OF NEWBORN INFANTS WITH PERINATAL BRAIN DAMAGE

M BECKER and K MENZEL

From the Department of Paediatrics Medical Academy Erfurt GDR

ABSTRACT Becker M and Menzel K (Department of Paediatrics Medical Academy Erfurt GDR) Brain typical creatinekinase in the serum of newborn infants with perinatal brain damage Acta Paediatr Scand 67 177 1978.—The normal values of serum CK activity in healthy newborns varied greatly (95% range 4–165 U/l 257 probands) and the borderline to pathological values was not sharp Brain typical CK isoenzyme (CK BB) could be detected in serum of 6 of 8 infants with perinatal brain damage by means of agar gel electrophoresis The combination of neuropathological symptoms increased total serum CK activity and significant amount of CK BB in serum seemed to be a sure sign of severe CNS-damage in newborns

KEY WORDS Creatinephosphokinase CK isoenzymes agar gel electrophoresis perinatal brain damage

Investigations of the serum activity of creatinekinase (CK) in cases of diseases of the CNS proceed from the assumption that in the presence of a lesion of the CK rich brain an increased enzyme leakage into the serum is to be expected as is the case in diseases of other CK rich tissues namely the heart and skeletal muscles

Belton et al (2) were unable to observe any increase of enzyme activity in the serum following *epileptic* grand mal seizures when a muscle relaxant had been administered to the patient previously In the period immediately following cerebral seizures meningoencephalitis cerebral vascular insufficiency head injuries perinatal hypoxia and in cases of acute psychosis only the muscular type of the CK isoenzymes (CK MM) could be ascertained in the serum using CK isoenzyme determination procedure (3 5 7) Seepage of the CK BB from a damaged area of the CNS into the cerebrospinal fluid could not be demonstrated even after repeated attempts (6 8 9 11 13 17)

All the more significant therefore are 1) The presence of brain typical CK BB in the serum of seriously asphyctic newborn infants by means of the ionic exchange chromatography procedure with DEAE sephadex (8) and 2) the demonstration of CK BB after cellulose column chromatography of serum of adults one day after craniotomy plus further neurosurgical procedures (12)

This study will examine whether a high level of serum CK activity of newborn infants with perinatal brain damage is due to seepage of the brain typical CK isoenzyme

METHODS

The isoenzyme determination by means of agar gel electrophoresis was performed essentially according to a procedure reported by Veen & Willebrands (16) Purified agar (Behring Rein Agar as well as Serva agar agar) was heated with destilled water and 0 05 M so-

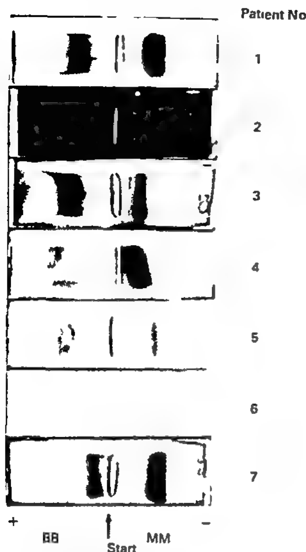


Fig 1 CK isoenzyme electropherograms of serum samples (no 1–6) and subdural fluid (no 7) of newborn infants with perinatal brain damage

dium-diethyl barbiturate/diethylbarbituric acid buffer pH 8 4 in a 1 1 ratio in a boiling water bath to a clear 1% solution To activate CK 0 28 ml of 2 mercaptoethanol was added to each litre of agar gel Microscopic slides were covered with the agar gel and an indentation of about 45 mm³ was made After 20 µl of undiluted serum had been inserted into the gel separation of the samples was achieved within 50–60 min at 40–45 A and 100–110 V (silver/silver chloride electrodes 0 05 M sodium diethyl barbiturate/diethylbarbituric acid buffer pH 8 4) at room temperature

After electrophoresis the isoenzymes were detected by means of a modification of the method employed by Rosalki (14 15) The concentration of the stain ingredients were as follows

Glucose	4 15 mM/l
ADP	0 83 mM/l
AMP	78 00 mM/l
NADP	0 33 mM/l
Creatine phosphate	2 10 mM/l
$MgSO_4$	18 70 mM/l
HK (280 000 U/l Boehringer)	1 mM/l
GPDH (140 000 U/l Boehringer)	1 mM/l
Nitroblue tetrazolium	3 26 mM/l
Phenazine methosulfate	25 00 mM/l
Mercaptoethanol	0 78 ml/l

After staining for 60 min at 37°C followed by thr[ee] hours of fixation in a solution of ethanol acetic acid an[d] water (15 1 4 v v v) the gels were rinsed in distill[ed] water for 24 h and dried in the air

CLINICAL MATERIAL

Serum samples from 19 newborn infants of normal a[nd] subnormal weights (classification was according t[o] Lubchenko s gestation age/weight curves) who showe[d] increased serum CK activity were analysed The age [of] the infants ranged from one to four days Eleven infan[ts] were affected by respiratory distress syndrom and [...] showed clinical evidence of perinatal brain damage It wa[s] difficult to decide whether the total CK activity is patho[-] logical or not as in healthy newborns the normal valu[e]

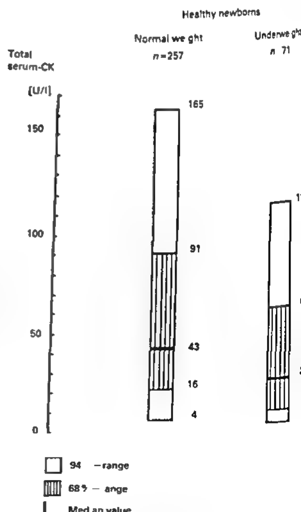


Fig 2 Total serum CK activity of normal weight an[d] underweight newborn infants n=sample size

Table 1 *Serum CK activity and CK isoenzymes in newborn infants with perinatal brain damage*
a=Serum n=normal weight b=haemorrhagic subdural fluid u=underweight

Patient no	total-CK (U/l)	CK MM	CK BB	Neuropathological findings	Autopsy findings of CNS
1 u	227 a	+++	+++	Seizures Respiratory disturbances Muscle hypotonia	Subdural bleeding Ganglion cell degenerations Edema
2 n	253 a	+++	+++	Seizures Respiratory disturbances Muscle hypotonia	Severe edema Ganglion cell degenerations
3 u	167 a	+++	+++	Respiratory disturbances Muscle hypotonia	Edema
4 n	214 a	+++	++	Respiratory disturbances	Edema
5 n	167 a	++	++	Muscle hypotonia	Edema
6 u	162 a	+	+	Seizures Muscle hypotonia	Edema
7 n	310 a	+++	—	Seizures	Extensive subdural bleeding
	296 b			Muscle hypotonia	

vary greatly and also depend among other things (18) on the body weight. The values shown in Fig. 1 were measured by a CK test set (Fermognost®) which uses the forward reaction of the enzyme. Blood samples were taken during the first 4 days of life. Within this period there were no significant changes in enzyme activity. After the blood samples had been taken they were stored in plastic tubes at +4 C and were analysed within 4 hours.

RESULTS AND DISCUSSION

In the serum samples of 11 newborns affected by respiratory distress syndrom only CK MM was found. Two newborns with clinical manifestation of brain damage and total CK activity of 197 U/l and 201 U/l respectively also had only CK MM in the serum. Fig. 2 shows the electropherograms of the serum samples of the other 6 infants with clinical manifestation of perinatal brain damage (see Table 1) in which not only CK MM but also CK BB was detectable. The CK BB band is somewhat blurred and moves towards the anode approximately as fast as the CK MM towards the cathode. Four of these children died within the first weeks after birth. In the case of patient no. 7 who died of meningorrhagia the haemorrhagic subdural fluid obtained by subdural puncture was separated and as in the serum only CK MM was found. However the possibility that the isoenzyme CK BB was converted into products of approximately CK MB or CK MM mobility in electropherogram nr. 7 cannot be excluded. The second dye band shown there is located near the start groove and could be the so-called MB like band observed by Cho et al. (4) after 72 h incubation of rabbit brain extract containing CK BB added to human plasma. The position of this dye band corresponds approximately to the albumin fraction and in many cases it could be observed in addition to a CK MM and/or CK BB band. For this reason and since in patient nr. 1 even 72 h after the sample had been taken the electropherogram was unchanged without any indication of convertion of mobility we consider it to be unspecific. The high CK BB intensity in electropherogram no. 1 and no. 2 in combination with the degeneration of the ganglion cells in these infants suggest that the evidence of CK BB in the serum is a direct indication of brain damage.

According to our results, which are in agreement with those of Menzel et al. (11) the following pattern

- neuropathological symptoms
- increased total serum CK activity
- significant amount of CK BB in serum

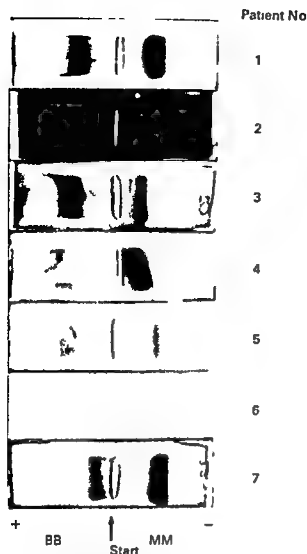


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HK (280 000 U/l Boehringer)	1 mM/l
GPDH (140 000 U/l Boehringer)	1 mM/l
Nitroblue tetrazolium	3 76 mM/l
Phenazine methosulfate	75 00 mM/l
Mercaptoethanol	0 78 ml/l

After staining for 60 min [illegible] 37°C followed by three hours of fixation in a solution of ethanol acetic acid and water (15 1 4 v v v) the gels were rinsed in distilled water for 24 h and dried in the air

CLINICAL MATERIAL

Serum samples from 19 newborn infants of normal and subnormal weights (classification was according to Lubchenko s gestation age/weight curves) who showed increased serum CK activity were analysed The age of the infants ranged from one to four days Eleven infants were affected by respiratory distress syndrom and showed clinical evidence of perinatal brain damage It was difficult to decide whether the total CK activity is pathological or not as in healthy newborns the normal values

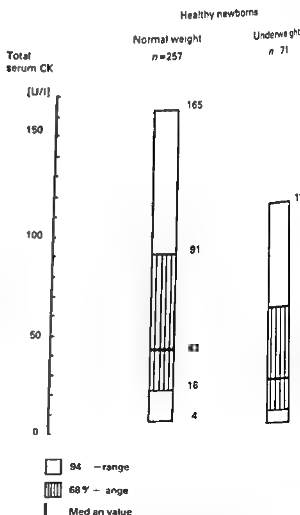


Fig 2 Total serum CK activity of normal weight and underweight newborn infants n = sample size

Table 1 *Serum CK activity and CK isoenzymes in newborn infants with perinatal brain damage*

a=Serum n=normal weight b=haemorrhagic subdural fluid u=underweight

Patient no	total CK (U/l)	CK MM	CK BB	Neuropathological findings	Autopsy findings of CNS
1 u	227 a	+++	+++	Seizures Respiratory disturbances Muscle hypotonia	Subdural bleeding Ganglion cell degenerations Edema
2 n	253 a	+++	+++	Seizures Respiratory disturbances Muscle hypotonia	Severe edema Ganglion cell degenerations
3 u	167 a	+++	+++	Respiratory disturbances Muscle hypotonia	Edema
4 n	214 a	+++	++	Respiratory disturbances	Edema
5 n	167 a	++	++	Muscle hypotonia	Edema
6 u	162 a	+	+	Seizures *Muscle hypotonia*	Edema
7 n	310 a	+++	—	Seizures	Extensive subdural bleeding
	96 b			Muscle hypotonia	

vary greatly and also depend among other things (18) on the body weight The values shown *in Fig 1 were meas*ured by a CK test set (Fermognost®) which uses the forward reaction of the enzyme Blood samples were taken during the first 4 days of life Within this period there were no significant changes in *enzyme activity After the blood* samples had been taken they were stored in plastic tubes at +4 C and were analysed within 24 hours

RESULTS AND DISCUSSION

In the serum samples of 11 newborns affected by respiratory distress syndrom only CK MM was found Two newborns with clinical manifestation of brain damage and total CK *activity of 197 U/l* and *201 U/l* respectively also had only CK MM in the serum Fig 2 shows the electropherograms of the serum samples of the other 6 infants with clinical manifestation of perinatal brain damage (see Table 1) in which not only CK MM but also CK BB was *detectable* The CK BB band is somewhat blurred and moves towards the anode approximately as fast as the CK MM towards the *cathode Four of these children* died within the first weeks after birth In the case of patient no 7 who died of meningorrhagia the *haemorrhagic* subdural fluid obtained by subdural puncture was separated and as in the serum only CK MM was found However the possibility that the isoenzyme CK BB was converted into products of approximately CK MB or CK MM mobility in electropherogram nr 7 cannot be excluded The second dye band shown there is located near the start groove and could be the so-called MB like band observed by Cho et al (4) after 72 h incubation of rabbit brain extract containing CK BB added to human plasma The position of this dye band corresponds approximately to the albumin fraction and in many cases it could be observed in addition to a CK MM and/or CK BB band For this reason and since in patient nr 1 even 72 h after the sample had been taken the electropherogram was unchanged without any indication of convertion of mobility we consider it to be unspecific The high CK BB intensity in electropherogram no 1 and no 2 in combination with the degeneration of the ganglion cells in these infants suggest that the evidence of CK BB in the serum is a direct indication of brain damage

According to our results which are in agreement with those of Menzel et al (11) the following pattern

- neuropathological symptoms
- increased total serum CK activity
- significant amount of CK BB in serum

is a sign of severe CNS damage and seems to indicate an unfavourable prognosis quo ad vitam

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ANTINUCLEAR ANTIBODIES IN JUVENILE CHRONIC ARTHRITIS

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ABSTRACT Permin H Hørbov S Wiik A and Knudsen J V (Immunological Laboratory University Clinic for Infectious Diseases Rigshospitalet Copenhagen and Hospital of Physical Medicine and Rehabilitation Hornbæk Denmark) Antinuclear antibodies in juvenile chronic arthritis Acta Paediatr Scand 67 181 1978.—One hundred patients with juvenile chronic arthritis (JCA) were studied with respect to granulocyte specific and organ nonspecific antinuclear antibodies (GS and ON ANA) in relation to clinical features of disease Seventy two were girls and 28 boys Sixty seven patients had IgG ANA 31 IgM 10 IgA 6 IgD 19 IgE and 35 had ANA which fixed complement C3 Sixteen of 17 sera containing IgG GS-ANA were from girls The prevalence of IgG GS-ANA increased with the number of joints affected No patient with the acute febrile type of the disease had IgG GS-ANA or C3 fixing ANA The prevalence of IgG ON ANA did not differ significantly in the mono- pauci- polyarticular and acute febrile types of JCA Patients showing clinical activity more frequently had IgG and IgM ANA and C3 fixing ANA The high titers of ANA were most often seen in girls Chronic uveitis occurred in 10 of the patients and IgG ANA were present in sera from all of these

KEY WORDS Juvenile chronic arthritis uveitis granulocyte specific and organ nonspecific antinuclear antibodies immunoglobulin classes

Juvenile chronic arthritis (JCA) is one of several chronic disorders of childhood with varying clinical and serological manifestations which have been widely suspected to be immunologically induced Abnormalities have been shown in the humoral as well as in the cellular immunological systems (1–5 7–10 12–15) Antinuclear antibodies (ANA) have been reported to occur in 3 to 60% of sera in JCA (1 3–5 7–10 12–15) The wide differences shown in the prevalence of ANA in these studies may result from differences in patient selection and in the assay system ANA may belong to all of the five immunoglobulin classes and may be capable of fixing complement (4 8 9 11 15)

The present study was undertaken to investigate the occurrence and titers of granulocyte specific and organ nonspecific (GS and ON) ANA in JCA patients and to correlate these findings to some clinical parameters

MATERIAL

Blood samples were obtained on occasions from 100 patients with JCA The patients were admitted to and followed at the Hospital of Physical Medicine and Rehabilitation Hornbæk All patients are fullfilling the criteria of Ansell & Bywaters (1) with the exception that synovial biopsy was not done in all mono- and pauciarticular cases

There were 72 girls with a mean age of 9 7 years (range 1 5–24) and 28 boys with a mean age of 10 6 years (range 1 4–22) The age at onset was defined as the age on appearance of the first symptom or sign consistent with the diagnosis JCA The types of JCA at onset were classified according to the manifestations during the first 3 months of the disease monoarticular having one joint pauciarticular having 2–4 joints and polyarticular having more than 4 joints involved The acute febrile onset

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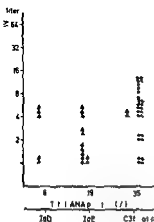


Fig 2 The prevalence and titers of IgD IgF and complement C3 fixing GS and ON ANA Symbols as in Fig 1

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The influence of sex is illustrated by the fact that 16 of 17 sera containing IgG GS ANA were from girls There was no significant difference in the incidence of any immunoglobulin class of ON ANA in girls versus boys The prevalence of IgG GS ANA increased with the number of joints affected at the onset of arthritis as well as the time of study (Table 2) None of the patients with the acute febrile type had IgG GS-ANA or complement fixing ANA and the titers of IgG ON ANA were low (≤32) The prevalence of IgG ON ANA was about the same in the 4 types of JCA at onset of the disease as well as the time of study

Compared to patients with inactive disease patients showing clinical activity more frequently had both IgG and IgM ANA and the ANA showed complement fixing properties though this trend was not statistically significant

Chronic uveitis occurred in 10 of the patients (9 girls and one boy) and IgG ANA were present in sera from all The onset type was 5 mono , 4 pauci and one polyarticular and at the time of study 9 were pauci and one was polyarticular The ANA titers varied considerably showing no relation to activity in the eye disease at the time of study

Twenty six of the patients had a family history of rheumatic diseases but these showed no increase in the incidence of ANA compared to the rest of the patients

In the control sera only 7 of 100 sera had positive ANA and all in low titers (≤32) Five had IgM ANA one of these GS ANA 2 had IgG and one IgA ANA none had IgD, IgE or complement fixing ANA

None of the patients with JCA showed positive LE cell tests and no clinical signs of amyloidosis were found in any patient

Five patients showed positive latex tests and 5 positive sheep cell agglutination reaction The mean age at onset of the disease in these patients was 9 1 and 10 5 years respectively Two patients who showed positive reactions both with latex reagent and the coated sheep cells had a late onset of the disease at 11 and 15 years respectively

Sixty two of the patients had elevated levels of serum IgG 17 had elevated IgM and 14

Table 2 *Relation of juvenile chronic arthritis type at onset and at time of study to the presence of IgG GS and ON ANA and complement C3 fixing ANA (%)*

Subjects	IgG GS ANA (%)	IgG ON ANA (%)	C3 fixing ANA (%)
Acute febrile type			
At onset ($n=13$)	0	46	0
At study ($n=5$)	0	40	0
Monoarticular type			
At onset ($n=39$)	10	6?	31
At study ($n=13$)	8	[illegible]	47
Pauciarticular type			
At onset ($n=29$)	14	6?	37
At study ($n=33$)	12	70	33
Polyarticular type			
At onset ($n=19$)	4?	78	58
At study ($n=49$)	2?	65	35

Table 1 *Relation of juvenile chronic arthritis type at onset and at time of study to the age and sex*

Subjects	At onset Mean age and range in years Total numbers (females/males)	At study Mean age and range in years Total numbers (females/males)
Acute febrile type	4 7 (1 1–14 3) 13 (5/8)	5 9 (1 4–14 5) 5 (2/3)
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showed persistent intermittent fever and lymphadenopathy and sometimes hepatosplenomegaly rheumatoid rash and joint involvement The classification at the time of study was based on the clinical condition during the last 3 months before serum sampling (Table 1)

In 86 patients the disease was active clinically as judged by joint swelling or two of the following features heat pain or tenderness possibly combined with limitation of motion In the remaining 14 cases there was no sign of clinical activity within the last 3 months

A control group of 100 individuals without chronic disease and without signs of joint disease was studied There were 49 females and 51 males with a mean age of 9 8 years (range 1 4–24)

METHODS

Sera were investigated for the occurrence of GS and ON ANA belonging to each of the 5 immunoglobulin classes and all were examined for complement C3 fixing properties Rat liver cryostat sections and smears of isolated and washed human leucocytes served as nuclear substrates (17 18 19) Sera were screened for IgG IgM and IgA ANA at dilution 1 16 and for IgD IgE and complement fixing ANA undiluted and 1 4 The latter dilution was used to avoid false negative reactions due to eventual prozone phenomena Positive samples were titrated by two fold dilutions using phosphate buffered saline (PBS) pH 7 2 as diluent The detailed immunofluorescence technique has been described earlier (17 18)

GS ANA are antibodies capable of reacting with nuclei of mature neutrophilic and eosinophilic granulocytes and monocytes *in vitro* but not with lymphocytes or other cell nuclei in this study represented by rat liver nuclei ANA are designated as granulocyte specific if they are found as the only detectable ANA in the specimen or if the specific reaction is seen at least 2 dilution steps higher than the lymphocyte and/or liver nuclear reaction Conversely ANA are called organ nonspecific if the lymphocyte and/or liver reactivity continues to the highest positive dilution of the specimen or at least 2 dilution steps lower than the granulocyte specific reaction

LE cell tests were carried out by the method of Hammer (6) Rheumatoid factors were demonstrated by the F II latex fixation slide test (Behringwerke West Germany) and Waaler Rose sheep cell agglutination test Titers of ≥32 and ≥40 respectively were considered abnormal

Serum concentrations of IgG IgM and IgA were determined by quantitative immunoelectrophoresis (16) Monospecific rabbit antisera were purchased from Dakopatts Denmark

Conjugates Fluorescein isothiocyanate (FITC)-labelled rabbit IgG specific for human γ μ and α chains and the β1c component of human C3 were from Dakopatts Rabbit antisera specific for δ and ϵ chains were from Behringwerke The isolated IgG fractions were labelled with FITC according to previously described methods (18) The anti immunoglobulin conjugates were tested for specificity by means of IgG IgM IgA IgD and IgE monoclonal bone marrow specimens from patients with multiple myeloma and macroglobulinaemia (18 19) All antisera showed monospecific reactions in crossed immunoelectrophoresis The fluorescein/protein ratios as estimated by OD 495/280 nm were 0 5–0 7 The slides were examined in a Leitz Orthoplan fluorescence microscope equipped for incident light illumination (17)

RESULTS

Seventy six of 100 JCA sera showed presence of at least one immunoglobulin class of ANA Sixty seven of the patients had IgG ANA 31 had IgM and 10 IgA ANA (Fig 1) In 27 patients the titers of IgG ANA were ≥256 These high titers were most often seen in girls The high titers were not correlated to clinical activ

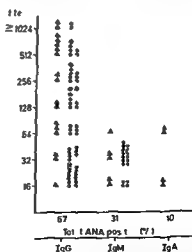


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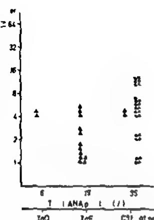


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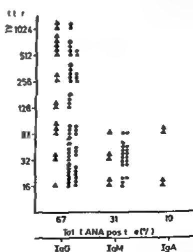


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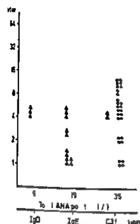


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Acute febrile type			
At onset ($n=13$)	0	46	0
At study ($n=5$)	0	40	0
Monoarticular type			
At onset ($n=39$)	10	67	31
At study ($n=13$)	8	69	47
Pauciarticular type			
At onset ($n=79$)	14	62	37
At study ($n=33$)	11	70	33
Polyarticular type			
At onset ($n=19$)	4	78	58
At study ($n=49$)	7	65	35

elevated IgA With one exception elevated IgM was only seen in girls Diminished levels of serum IgM and IgA were only found in one and 2, respectively Diminished levels of IgG were not found

DISCUSSION

Our study confirms previous reports which have shown a relation between the clinical type of JCA at the time of onset and the age of the patients (3 5 14) About two thirds of the children had mono or pauciarticular type at the onset and the frequency of the acute febrile type and polyarticular type onset was about equal More boys than girls had an onset of the acute febrile type (Table 1) It also emphasizes that polyarticular disease eventually develops in the majority of the patients (5 14)

The higher incidence of serum IgG ANA reported here does not reflect a longer duration of disease in our patients since analysis shows a similar incidence for patients with a short disease duration (13 14) The high percentage of positive sera may reflect a higher sensitivity of the test employed (17)

As previously shown (9 13) we found a higher incidence of IgG GS ANA in girls than in boys IgG GS ANA were most commonly found in patients with many affected joints (Table 2) Patients with a high titer of IgG ANA were most often girls as shown also by Rudnicki et al (14) Our data on GS ANA in relation to clinical activity were too scarce to allow any conclusion however no relation was found in the study by Rosenberg et al (13) Since this latter group of investigators did not study ON ANA worked with lower dilution of test sera and used a polyspecific conjugate our study can not be directly compared to theirs

Both GS and ON ANA were found to be heterogeneous as regards their immunoglobulin class nature in that they existed in all 5 classes

The patients with clinical disease activity more frequently showed presence of IgG and IgM ANA and ANA possessing complement fixing properties

The findings of increased levels of serum IgG and sometimes IgM and IgA confirms previous reports (2 5 14)

ANA in sera from patients with adult rheumatoid arthritis are different from ANA in JCA in several respects In adult rheumatoid arthritis a higher incidence of IgG GS ANA is found and IgM ANA occur much more frequently often in high titers (13 17) The higher prevalence of IgG GS ANA may be due to involvement of more joints in the adult form The lower prevalence and titers of IgM ANA in JCA may somehow relate to the fact that IgM rheumatoid factors are rarely found in these patients (2 3 5 7 10 14) The patients who had rheumatoid factors as shown by one or both of the tests employed all had a late onset of the disease were of the polyarticular type and all but one were girls However some similarities seems to exist between the adult and juvenile form of rheumatoid arthritis since 15 out of 19 sera from JCA revealed IgE ANA with granulocyte specificity and similar findings have been obtained in adult rheumatoid arthritis (11)

IgG ANA is a pathological feature which can be seen in many diseases However when the diagnosis of JCA is established the finding of a positive IgG ANA reaction may have prognostic significance for a development of chronic uveitis as all 10 patients in this study had positive IgG ANA (3 5 15)

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Acta Pædiatr Scand 67 187–192 1978

ANO RECTAL MANOMETRY IN THE DIAGNOSIS OF HIRSCHSPRUNG S DISEASE IN INFANTS

B FRENCKNER

From the Department of Paediatric Surgery St Goran s Hospital Stockholm Sweden

ABSTRACT Frenckner B (The Department of Paediatric surgery St Goran s Hospital Stockholm Sweden) Ano-rectal manometry in the diagnosis of Hirschsprung s disease in infants Acta Paediatr Scand 67 187 1978 —Manometric recordings of internal sphincter activity were performed during distension of the rectum in 10 healthy control infants and in 9 infants with clinical signs of Hirschsprung s disease In 8 of the healthy infants relaxations of the internal sphincter were obtained which were maximal 4 to 7 secs after rectal distension This was also the case in 5 of the patients who were later proven not to have Hirschsprung s disease In 3 patients no relaxations of the internal sphincter could be recorded Subsequent rectal biopsy revealed absence of ganglion cells confirming the diagnosis Hirschsprung s disease The remaining 3 infants (? controls and 1 patient) could not be calmed during the examination and the results were inconclusive It is concluded that ano-rectal manometry is a valuable method of examination in the diagnosis of Hirschsprung s disease in infants No false results were obtained in this study Furthermore it is an easy procedure without risk or discomfort for the patient

KEY WORDS Megacolon constipation defecation ano-rectal manometry

The internal anal sphincter is the distal part of the circular enteric muscular coat Like other smooth muscle it is normally in a state of tonic contraction and keeps the anal canal closed When the rectum is distended however there is a transient relaxation of the internal sphincter (2 8 12) which results in a fall of anal pressure This reflex also occurs in patients with flaccid paraplegia due to sacral nerve root damage which indicates that it is mediated via local nerve plexuses in the gut When the ganglion cells of the rectal wall are absent however as in Hirschsprung s disease the internal sphincter fails to relax upon rectal distension (10 11) Several authors have found this to be a valuable diagnostic test of Hirschsprung s disease (1 9 13 14 15)

The aim of the present investigation was to further study the activity of the internal anal sphincter in healthy infants as well as in those with clinical signs of Hirschsprung s disease

METHODS

Equipment Anal pressure was recorded at the level of the internal sphincter using the cuff of a Portex endotracheal tube no 5 0 The outer end of the tube was cut so that its total length became 13–14 cm and the top of the cuff was tied with a silk ligature to reduce its length to 1 0–1 5 cm (Fig 1) The cuff was then filled with water and via a thin polyethylene tube connected to the recording equipment Finally the amount of water was adjusted so that the cuff was expanded but the pressure inside it did not exceed zero

Rectal distension was achieved with a latex balloon When empty this measured 1 5× . cm It was connected to a polyethylene tube about 50 cm long with an internal diameter of ? 0 mm and an external of 2 4 mm (Fig 1) This tube led to the recording equipment via a three way stopcock through which air could be inflated into the balloon

As recording equipment a pressure transducer (Statham P 23) amplifier (Grass 7 P1) and recorder (Grass 7)

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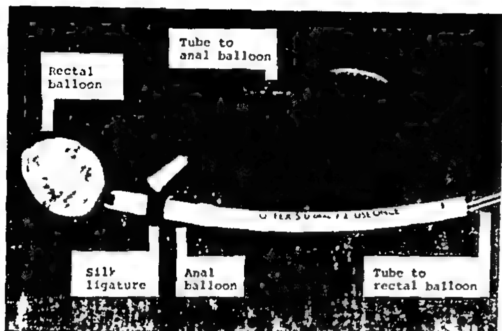


Fig 1 To record anal pre sure the cuff of a Portex e dotracheal tube is used T top of the cuff is tied with silk ligature in order to re duce its length to 1 0–1.5 c The tube to the rectal bal loon passes through the e dotracheal tube

were used separately on each line from the rectal balloon and the anal cuff respectively (Fig 2)

Procedure The subjects were lying supine The rectal balloon was first placed in position with the aid of some exploration cream and a swab To ensure that the balloon lay unfolded it was inflated with 10 ml air which was immediately evacuated The anal balloon (i e the cuff of the endotracheal tube) was then placed just inside the anal verge with the tube to the rectal balloon running through it (Fig 2) An assistant held this balloon in position during the examination

Anal pressure was recorded continuously When it had stabilized the rectal balloon was inflated with air in portions from 5 ml to 20 ml This was repeated several times Each inflation took less than 0 5 secs and the rectal balloon was kept filled each time for about 10–20 secs before it was evacuated

During each examination great care was taken to keep the child quiet and calm Mostly the mother was present A feeding bottle or a dummy was often given and especially the younger children were mostly satisfied with this In some instances the infants were premedicated with diazepam (Valium Roche) 0 3 mg/kg body weight given orally one hour before the examination

Data in the text are given as mean±S D unless otherwise stated

MATERIAL

The study was undertaken on 10 healthy infants aged 2 weeks to 10 months (control group) and on 9 patients aged 1–12 months (patient group) (Tables 1 and 2) None of the controls had any history of ano rectal disorders diarrhoea or constipation The 9 patients had been admitted to the hospital because of clinical signs of Hirschsprung s disease They presented with severe constipation sometimes combined with poor weightgain or abdominal distension and in two cases with intestinal obstruction (Table 2) In all cases the final diagnosis was unknown at the time of this examination

RESULTS

Control group 8 of the 10 healthy contro were quiet during the investigation and re istrations free from artefacts were obtain (Table 1) In these 8 controls anal pressu stabilized after a few minutes and average 47±18 mmHg In response to a rectal diste sion with 10 ml a fall of anal pressure due relaxation of the internal sphincter was r corded in all of them A typical record shown in Fig 3 The relaxation was maxim at an average of 6 secs (range 4–7) after t rectal inflation *With 5 ml in the rectal balloo* relaxations of the internal sphincter were r corded in six of the controls (mean time 5 sec range 4–6) After the relaxation anal pressu

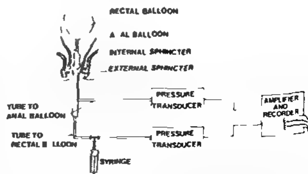


Fig 2 Diagrammatic representation of the method wi an anteroposterior view of the rectal and anal balloons i situ

Table 1 *Age weight and results of ano rectal manometry in the ten healthy controls*

Patient	Age (months)	Weight (g)	Preme dicated	Defe cation	Anal pressure (mmHg)	Response to rectal distension (10 ml)
S Y	[illegible]	3 045	–	+	25	Relaxation
Go S	[illegible]	4 010	–	+	48	Relaxation
T L	[illegible]	3 950	+	–	40	Relaxation
P A	1	3 850	+	+		Crying during the examination
Go E	[illegible]	2 610	+	+	35	Relaxation
M W	2	4 400	–	+	62	Relaxation
K S	6	7 510	–	–		Crying during the examination
M T	7	7 660	–	–	50	Relaxation
M L	9	8 550	+	+	83	Relaxation
C I	10	9 300	–	–	35	Relaxation

rose and mostly reached its resting level while the rectal balloon was still expanded

Two of the 10 controls could not be kept quiet one despite premedication and no stable level of anal pressure was obtained This made it difficult to detect relaxations of the internal sphincter in response to rectal distension Relaxations were in fact suspected a few times in both of them but the registrations could not be considered conclusive

Six of the ten controls defecated during the examination (Table 1) This was invariably preceded by a fall of anal pressure to 0–10 mmHg and this sometimes lasted for several minutes After the defecation—or sometimes after several defecations—anal pressure returned to its resting level

Patient group 8 of the 9 patients with suspected Hirschsprung s disease were quiet and conclusive registrations were obtained (Table 2)

In 3 patients no relaxation of the internal sphincter was detected when the rectum was expanded with 5–20 ml air This is demonstrated in Fig 4 None of them defecated during the examination Mean anal pressure at rest was 51 mmHg (range 30–62) In view of this absence of internal sphincter relaxations

Table 2 *Age weight symptoms and results of ano rectal manometry in the nine patients with clinical signs of Hirschsprung s disease*

Patient	Age (months)	Weight (g)	Problem	Preme dicated	Defe cation	Anal pressure (mmHg)	Response to rectal distension (10 ml)
P M	[illegible]	4 530	Constipation	+	–	[illegible]	Relaxation
Je E		5 180	Constipation	+	+	75	Relaxation
P A	6	4 310	Intestinal obstruction	–	+	50	Relaxation
Ji E	7	6 160	Constipation	+	–	58	Relaxation
J W	8	7 700	Constipation	+	–		Crying during the examination
A D	12	6 890	Constipation	+	–	50	Relaxation
P E	1	3 70	Constipation abdom distension	–	–	30	No relaxation
J O	3	4 170	Constipation	–	–	6	No relaxation
O K	5	5 600	Intestinal obstruction	+	–	60	No relaxation

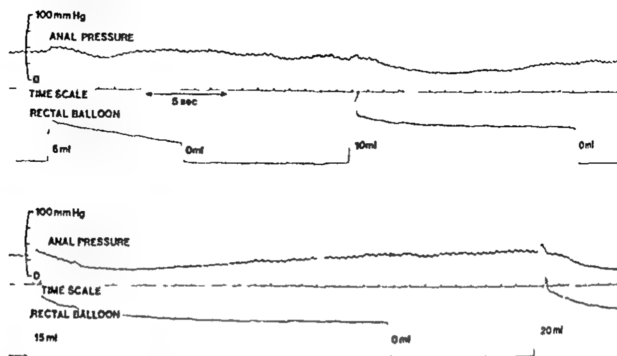


Fig 3 Recording of anal pressure during rectal filling in a healthy girl one month old (T L) In response to each inflation of the rectal balloon there is a clear fall of pressure due to a relaxation of the internal sphincter

these three patients were considered to have Hirschsprung s disease The diagnosis was subsequently confirmed by a histological examination which revealed an absence of ganglion cells in the rectal wall

In another 5 patients relaxations of the internal sphincter were recorded in response to rectal distension with 10 ml (Table 2 Fig The relaxations were maximal at an average 6 secs (range 4–7) after the rectal inflation

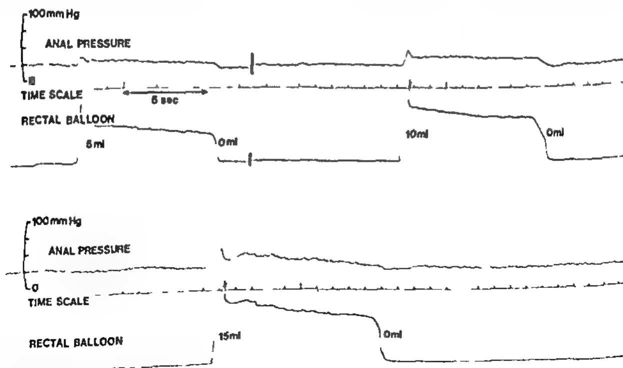


Fig 4 Recording of anal pressure during rectal filling in a 25 day old boy with constipation and abdominal distension (P F) No relaxations of the internal sphincter were recorded Subsequent rectal biopsy revealed absence ganglion cells

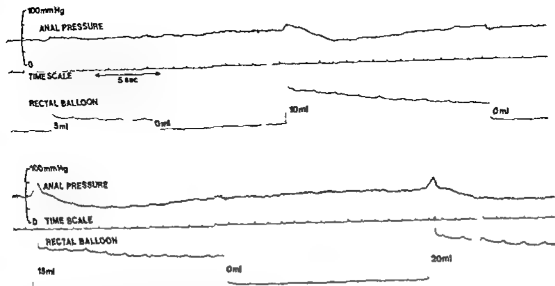


Fig 5 Recording of anal pressure during rectal filling in a 6-month-old boy with intestinal obstruction (P A) With 10 ml or more in the rectal balloon clear relaxations of the internal sphincter are seen Subsequent operation re vealed peritoneal adhesions which obstructed the bowel

Two of these patients defecated during the examination Anal pressure at rest averaged 49±14 mmHg The patients were followed up 4–9 months after the examination at which time there was no clinical suspicion of Hirschsprung s disease in any of them

One patient (J W) could not be calmed during the manometry making it difficult to obtain a satisfactory recording Relaxations of the internal sphincter were suspected but the examination could not be considered fully conclusive When followed up 5 months later the patient was healthy and no longer troubled with constipation

DISCUSSION

The pressure which can be recorded in the anal canal at rest is generated predominantly by the internal sphincter (3 7) although the external sphincter exhibits continuous tonic activity (5) Contractions of the external sphincter result in great increases in anal pressure (7) Consequently anal pressure recorded at the level of the internal sphincter in a quiet calm child largely reflects the activity of this sphincter But when the child is irritated or crying the external sphincter contracts irregularly thereby influencing anal pressure which no longer reflects internal sphincter activity alone It is therefore essential that the child is contented and not crying during the examination

In this investigation altogether 19 infants were examined Sixteen of them were quiet and calm enough to give satisfactory registrations of anal pressure free from confusing external sphincter activity

Anal pressure at rest did not differ between healthy infants (47±18 mmHg) patients with Hirschsprung s disease (51±18 mmHg) or the other constipated infants (49±14 mmHg) This finding agrees with the results of Aaronson & Nixon (1)

In response to rectal distension in healthy infants anal pressure falls due to a relaxation of the internal sphincter as in adults (2 8) A volume of 10 ml was found to be sufficient to elicit the relaxation reflex which was maximal between 4 and 7 secs after the inflation After this anal pressure began to rise that is the internal sphincter increased its tone while the rectum was still distended Consequently if

the maximal fall of anal pressure occurs immediately upon inflation of the rectal balloon or if anal pressure does not begin to rise before the balloon is evacuated the response should be interpreted not as a normal relaxation of the internal sphincter but as an artefact This might be the case if the rectal balloon is not properly in situ but lies folded beside the anal tube

In the patient group registrations regarded as conclusive were obtained in 8 of the 9 cases The 3 patients in whom no relaxations of the internal sphincter could be elicited were subsequently proven to have Hirschsprung s disease In the other 5 patients relaxations were recorded Rectal biopsy was not undertaken in these cases but the patients were followed up and their clinical course excluded Hirschsprung s disease Ano rectal manometry consequently gave the right diagnosis in all these cases

Half of the infants without Hirschsprung s disease defecated during the examination Just before this there was a period with very low anal pressure that is the internal sphincter was relaxed No such period of low pressure was noted in any of the patients with Hirschsprung s disease and none of them defecated This may indicate that reflex defecation is not induced by ano rectal manometry in patients with Hirschsprung s disease One would in fact expect this in view of their inability to relax the internal sphincter

It is wellknown that the diagnosis of Hirschsprung s disease may be difficult to establish Barium enema examination usually demonstrates a narrow distal segment but the interpretation is difficult particularly in (*a*) patients with a very short segment (*b*) patients with involvement of the entire colon and (*c*) patients with a colostomy (4) Conventional rectal biopsy sometimes proves technically difficult but is usually highly reliable It is however not without complications and mortality (6) Consequently ano rectal manometry must be recommended as a diagnostic aid for Hirschsprung s disease It is a simple examination and involves no risk or discomfort for the patient A high diagnostic reliability has been demonstrated in the present investigation in accordance with several other studies (1 9 13 14 15)

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PHENOBARBITAL METABOLISM IN ADULTS AND IN NEWBORN INFANTS

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ABSTRACT Boreus L O Jalling B and Kållberg N (Departments of Clinical Pharmacology and Paediatrics Karolinska Hospital and the Karolinska Pharmacy Stockholm Sweden) Phenobarbital metabolism in adults and in newborn infants Acta Paediatr Scand 67 193 1978 —Two adult volunteers and four newborn infants were given a *single dose of phenobarbital* The output in the urine of unchanged phenobarbital and of the two main metabolites *p* hydroxy phenobarbital and conjugated *p* hydroxy phenobarbital was followed during 8 days in the newborns and during 2 or 4 weeks in the adults The plasma levels were also determined and some pharmacokinetic constants calculated It was found that the newborn patients excreted unchanged phenobarbital and *p* hydroxy phenobarbital in the same proportions relative to dose as did the adult volunteers i e 16–17% unchanged drug and 9–10% of the metabolite during the first 8 days after administration On the other hand there was a clear-cut age difference in output of conjugated metabolite where the newborns excreted only 5% of the given dose during the 8-day observation period The corresponding value for the adults was [illegible]% It is concluded that a poor conjugating capacity in the newborn may not have any serious consequences with a drug like phenobarbital where major alternative routes of excretion exist (unchanged drug and unconjugated metabolite) The clinical significance of immature drug metabolism in the newborn cannot be determined unless the excretion of all major metabolites and of unchanged drug is taken into consideration

KEY WORDS Developmental pharmacology drug metabolism neonatal hyperbilirubinemia phenobarbital

Newborn infants may be exposed to a variety of drugs either as part of neonatal therapy or as a result of obstetrical needs or treatment of maternal disease Since biotransformation of a drug is often a main determinant for the intensity duration and termination of the pharmacological effect it is essential to define the ability of the newborn to metabolize clinically used drugs

However only scattered quantitative data are available on drug metabolism in the newborn Studies in which prenatally administered drugs have been recovered as such or as metabolites in neonatal urine do not give true information on metabolic capacity since maternally produced metabolites cannot be separated from those arising in the baby itself

Therefore metabolic studies of drugs given directly to the newborn are usually more fruitful from the quantitative point of view For ethical reasons however they must be limited to drugs needed in neonatal care

In the present investigation the use of phenobarbital for treatment of convulsions of the neonate has been exploited for determination of plasma concentrations and urinary output of phenobarbital and its metabolites fol

Table 1 *Clinical data*

	Age	Length of gestation (weeks)	Birth weight (g)	Diagnosis
E B ♀	36 y	–	–	Voluntary subject
A L ♂	30 y	–	–	Voluntary subject
Pat 1 ♂	41 h	41	4 050	Moderate asphyxia intracranial bleeding convulsions
Pat 2 ♂	74 h	41	3 100	Moderate birth asphyxia convulsions
Pat 3 ♂	49 h	40	3 930	Severe birth asphyxia suspected seizures
Pat 4 ♂	7 h	41	3 680	Severe birth asphyxia bronchopneumonia suspected seizures Died at 8 days of age

lowing single oral or intramuscular doses The same measurements were performed on two adult healthy volunteers

MATERIAL AND METHODS

Patients and volunteers Four male newborn infants were included in the study They were given single doses of phenobarbital as part of treatment for seizures at the age of 7 to 74 hours Clinical data on these patients are given in Table 1 and in the legends to Figs 3–6

Two healthy adults one female and one male volunteered to take a single oral dose of phenobarbital Plasma and urine were then collected for a period of one month and of two weeks respectively Data on the subjects are given in Tables 1 and 2

Drug Commercially available phenobarbital (Fenem ACO Co) was used as 15 or 50 mg tablets For injecti the commercial preparation was diluted to a concentrati of 20 mg phenobarbital The solvent contained propyle glycol (40 g/100 ml water) The pH was 8 5–9 5 Dos and routes of administration are shown in Table 2

Blood sampling Capillary blood was taken usi heparinized routine hematocrit tubes by finger tip or he puncture The tubes were centrifuged and the plas stored frozen until analysis One control sample was a ways taken before the administration of the drug Plas was then collected once or twice daily throughout t observation period with the exception of more freque sampling during the first 24 hours in the newborns

Urine sampling Glued-on plastic bags were used f collection of urine in the newborns To minimize sk irritation the bag was emptied through a feeding tub inserted through the wall of the bag Emptying was ma as soon as a portion of urine had been voided The b was thus only exchanged if it did no longer stick to the ski The volunteers measured all voided urine portions A samples of urine both from the newborns and from th volunteers were kept frozen until analysis

Chemical assay The gas chromatographic metho developed for determination of phenobarbital in plasm and of phenobarbital and its two main metabolites urine have been described previously (4 5) The analytic procedure for urine has been slightly modified the ste column has been replaced by a 6 feet 1/8 glass colum filled with 3% OV 17 on Gas Chrom Q support 100/1 mesh the O_2 flow rate has been reduced to 200 ml/mi All analyses were performed in duplicate

RESULTS

All results of plasma and urine analyses ar presented graphically in Figs 1–6 Fig demonstrates the cumulative urinary output o phenobarbital and its main metabolites uncon jugated and conjugated *p* hydroxy phenobarbi tal in the neonates and in the adults Calcu

Table 2 *Pharmakokinetic data*

	Body weight (at time of administration)	Dose (mg/kg)	Disposition rate constant (hours $^{-1}$)	Half life (h)	Apparent conc at time 0 (µg/ml)	Apparent volume of distribution (l/kg)
E B ♀	48 kg	5 21 (oral)	0 0059	117	10 0	0 52
A L ♂	75 kg	4 33 (oral)	0 0082	84	9 3	0 47
Pat 1	4 180 g	12 0 (i m)	0 0113	61	20 2	0 59
Pat 2	2 910 g	20 6 (oral)	0 0084	[illegible]	21 0	0 98
Pat 3	3 940 g	11 4 (i m)	0 0058	119	17 8	0 64
Pat 4	3 680 g	12 0 (i m)	0 0040	173	18 1	0 66

$$\text{Apparent volume of distribution} = \frac{\text{dose (mg/kg)}}{\text{apparent concentration at time 0 (µg/ml)}}$$

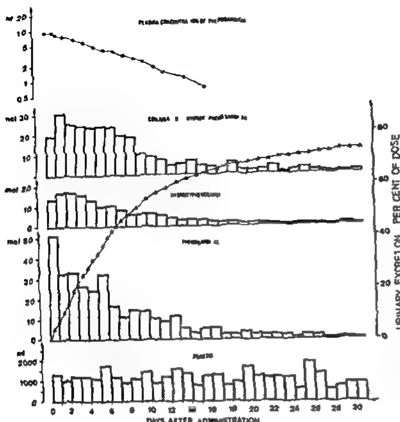


Fig 1 Plasma concentrations of phenobarbital and urinary excretion of phenobarbital and two of its metabolites during one month after a single oral dose (5 2 mg/kg) in the female subject E B Triangles denote cumulated excretion of unchanged and metabolized drug expressed as per cent *µmoles of the given dose*

lated pharmacokinetic data are given in Table 2

In the female subject E B (Fig 1) all urine during one month after the administration was collected and analysed The plasma concentration of phenobarbital had decreased below the sensitivity of the gas chromatographic method (0 5 µg/ml) after 17 days but both the drug itself and its two main metabolites could be found in the urine throughout the entire period of observation (31 days) During this time more than 70% of the oral dose was recovered in the urine as phenobarbital or metabolites

In the male subject A L (Fig 2) plasma and urine were collected during two weeks On two occasions (day 5-6 and 11) increased diuresis was produced by extra intake of fluid It was found that the daily recovery of unchanged phenobarbital in the urine was to some extent correlated to the diuresis this was not the case with the metabolites The pH was measured in some of the urinary portions and found to vary in subject A L between 5 0 and 7 0 Within this interval no correlation between urinary pH and output of unchanged phenobarbital could be demonstrated

From the cumulative plot (Fig 7) it is evident that both healthy adults and sick newborns can hydroxylate and conjugate phenobarbital and also excrete substantial amounts of this drug in unchanged form The renal excretion both of phenobarbital and its p hydroxylated metabolite was not inferior in the babies on the contrary patients no 1 and 2 well matched or even surpassed the adult volunteers

A definite age difference on the other hand exists in output of conjugated material (Fig 7C) Here all newborns show very low values

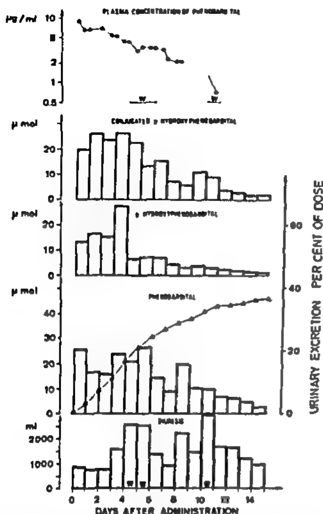


Fig 2 Male subject A I given a single oral dose of phenobarbital (4 3 mg/kg) Results of plasma and urine analyses presented as in Fig 1—Forced water diuresis (w) was produced on days 5–6 and 11

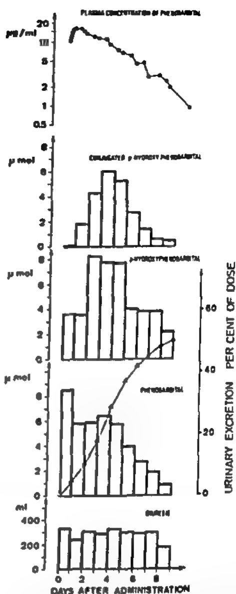


Fig 3 Newborn patient no 1 Full term infant slow progress during last part of delivery caused application of vacuum extractor After resuscitation regular breathing at the age of 15 min Convulsions on second day of life (opisthotonus clonic movements in left arm and leg) disappeared seven hours after administration of phenobarbital (12 0 mg/kg i m) Considered neurologically normal at the age of two weeks

during the first 3 days Only one of the babies (no 1) could compensate for this handicap during the observation period of 8 days (Fig 7 C)

DISCUSSION

The metabolism of phenobarbital has been the subject of some earlier studies Thus Butler (2) suggested that hydroxylation is of major importance for the inactivation of the drug and demonstrated the presence of *p* hydroxy phenobarbital in the urine of two men Almost half of this product was present in the unconjugated form The same metabolite was found by Algeri & McBay (1) in the urine from two subjects 54 and 80% appeared in the unconjugated form Ravn Jonsen et al (7) also found *p* hydroxy phenobarbital in the urine of a patient who was treated with high doses of phenobarbital

The nature of the conjugate is still unclear Butler (2) using glucuronidase was not able to demonstrate a glucuronic metabolite and assumed the existance of a sulphate conjugate of *p* hydroxy phenobarbital in man On the other hand our own studies (5) on phenobarbital

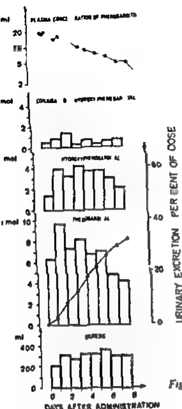


Fig 4

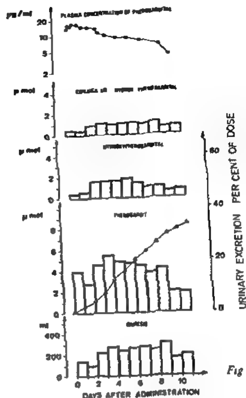


Fig 5

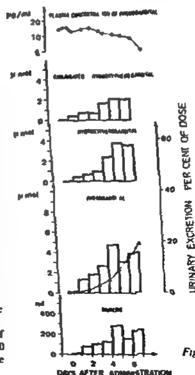


Fig 6

Fig 4 Newborn patient no 2 Full term infant in spite of uneventful delivery Apgar score was 2 three minutes after birth Ten minutes after birth all vital functions were considered satisfactory Seizures (chewing twitchings around the eyes clonic movements of the right hand) appeared at the age of ? 5 days and disappeared nine hours after administration of phenobarbital (?0 mg/kg orally) EEG ninth day of life revealed epileptogenic activity and long term antiepileptic treatment was instituted at two weeks of age

Fig 5 Newborn patient no 3 Full term infant with severe birth asphyxia breech presentation Seizures were suspected but never confirmed to be the expression of paroxysmal electric brain activity Phenobarbital (11 4 mg/kg i m) had no effect on the condition of the patient

Fig 6 Newborn patient no 4 Full term infant Severe birth asphyxia breech presentation Developed bronchopneumonia and died 8 days of age Suspicion of seizures caused administration of phenobarbital (1? 0 mg/kg i m) at the age of 7 hours but did not improve the condition of the patient

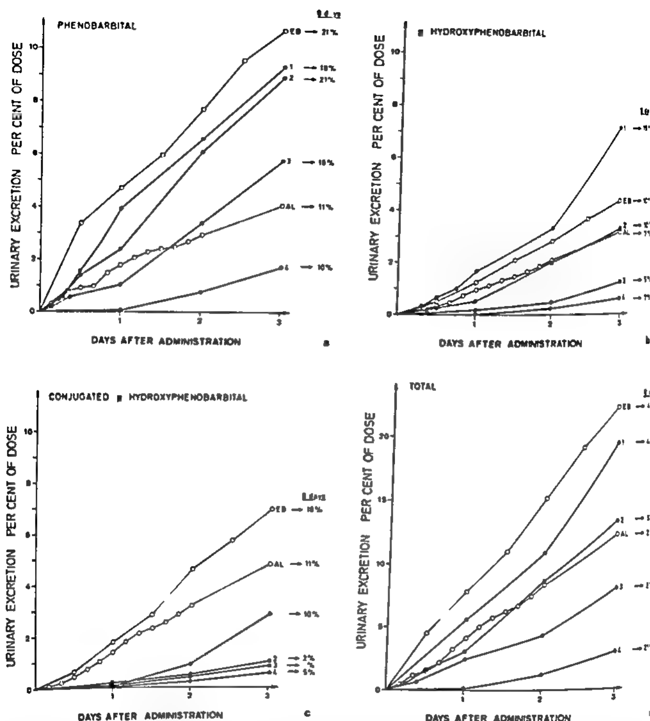


Fig 7 Cumulated urinary output (in per cent of given μmolar dose) of (A) Phenobarbital (B) *p* hydroxy phenobarbital (C) conjugated *p* hydroxy phenobarbital and (D) total material (phenobarbital and metabolites) in the two adult volunteers E B and A L and in the four newborn infants Values for the first three days following administration are shown as well as the cumulated per cent values for the 8th day

metabolites provided strong evidence that *p* hydroxy phenobarbital is conjugated in man mainly with glucuronic acid whereas no evidence for a sulphate conjugate was found

The present study seems to be the first where both plasma and urine have been analysed for an extended period after phenobarbital ingestion and with quantitative techniques Both our adult subjects excreted unchanged drug as well as conjugated and unconjugated *p* hydroxy phenobarbital in the urine during the entire observation period (4 and 2 weeks respectively) The cumulative output of material was somewhat higher in the

female than in the male subject 65 and 35% respectively of the given dose appeared in the urine during the first two weeks The female subject excreted about 75% of the single dose *during the 4 week period of urine sampling* In both volunteers the major part was recovered as phenobarbital and less than half in the form of metabolites The difference in total output between the two subjects could be mainly attributed to the large difference in output of unchanged drug this was much bigger in the female than in the male subject This difference could not be due to differences in urinary flow since the mean diuresis under basal conditions were about equal Such a comparison seems justified since the plasma levels of phenobarbital were similar

Of the two metabolites the conjugated *p* hydroxy phenobarbital dominated This conjugated *metabolite was usually present in the* urine in about twice as high amounts as unconjugated *p* hydroxy phenobarbital

The pattern of excretion in the newborn patients during the first 8 days after dose was remarkably similar to that of the adult volunteers Thus unchanged phenobarbital and *p* hydroxy phenobarbital appeared in newborn urine in the same proportions relative to dose as in adult urine The mean value for output of unchanged drug during this time period was 16% of the given dose for the two adults and 17% for the four newborns The corresponding values for *p* hydroxy phenobarbital was 9% and 10% respectively

*However if the renal excretion of the con*jugated metabolite is compared in the same way a clear-cut difference appears As mentioned above adults were found to produce twice as much conjugated as unconjugated metabolite In the newborn it is quite the reverse Only 5% of the given dose was excreted in conjugated form during 8 days compared to 15% in the adult This is a strong age difference but is only a moderate handicap for the total elimination of phenobarbital in the newborn period since the output of unconjugated metabolite and of unchanged drug is excellent The total amount of phenobarbital material during 8 days was 32% of dose in the newborn and 39% in the adults It is possible that the difference would have become even smaller if the observation period had been further extended This was however technically and ethically impossible

The poor ability of the newborn to conjugate seems to be the main handicap in drug metabolism in this age period There is very little quantitative data on this point in the literature but one early report is relevant viz the study by Vest in 1958 (8) He examined the fate of acetanilide by following the appearance of metabolites in the urine on a quantitative basis and found that the main metabolite glucuronide conjugated *p* aminophenol was excreted in much smaller amounts than is usually found in the adult

*The clinical significance of deficient conju*gation is dependent upon which alternative routes for elimination that exist for the drug in the given situation Also many different enzyme systems are involved in conjugation reactions In healthy full term newborns it was observed that the rate constant for acetaminophen glucuronide was smaller than in adults but that this handicap was compensated to some extent by a well developed capability for sulfate conjugation (6) An uncompensated conjugation deficiency combined with too high dose may lead to disastrous effects as is well known for chloramphenicol (for review see Done (3)) For phenobarbital as shown in the present investigation the low production of conjugated metabolite is so well compensated by the efficient output of non conjugated metabolite and of unchanged phenobarbital that the total renal elimination of the administered drug is almost as rapid in the sick newborns as it is in healthy adults

It is interesting to note that the clinical condition of the newborn seems to be an important determinant factor for drug metabolism perhaps more important than age differences in the case of phenobarbital The

newborns no 1 and 2 who were in a much better clinical condition than no 3 and 4 generally had a more efficient output of metabolites In baby no 4 probably the young age (7 hours) and the poor clinical state contributed to the low capacity to get rid of the administered dose Immaturity of kidney functions does not seem to be an explanation for low output of conjugate since unchanged phenobarbital with much less water solubility was readily excreted

ACKNOWLEDGEMENTS

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STUDIES ON A PATIENT WITH IN VIVO EVIDENCE OF TYPE I GLYCOGENOSIS AND NORMAL ENZYME ACTIVITIES IN VITRO

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ABSTRACT Chalmers R A Ryman B E and Watts R W E (Division of Inherited Metabolic Diseases MRC Clinical Research Centre Watford Road Harrow HA1 3UJ and Department of Biochemistry Charing Cross Hospital Medical School Hammersmith London U K) Studies on a patient with in vivo evidence of type I glycogenosis and normal enzyme activities in vitro Acta Paediatr Scand 67 201 1978.—Biochemical and clinical studies on a patient with hepatic glycogen storage disease are reported The patient showed many of the clinical and biochemical features of type I glycogenosis (glucose-6-phosphatase deficiency) but had normal activities of the following enzymes in liver tissue glucose-6-phosphatase (EC3 1.3 9) amylo-1 6-glucosidase (EC3 2 1.33) glycogen phosphorylase (EC2 4 1 1) fructose-1 6-diphosphatase (EC3 1.3 11) The urinary excretion of 2-oxoglutaric acid was greatly increased in this patient and in a case of enzymologically proven type I glycogenosis Abnormal 2-oxoglutaric aciduria has not been previously reported in the glycogen storage diseases The results are discussed in relation to the possible nature of the underlying biochemical defect in patients of this type

KEY WORDS Glycogen storage diseases glycogenosis type I variant enzymes 2-oxoglutaric aciduria

The known pathways of glycogen synthesis and degradation and the position of the enzyme deficiencies which are associated with the different types of glycogenosis (glycogen storage diseases) are shown in Fig 1 These diseases are rare and have a combined incidence of between about 1 in 40000 and 1 in 70000 (13 17) Type I glycogenosis [congenital deficiency of glucose-6-phosphatase (EC3 1 3 9)] is one of the more common types but there are occasional cases of excessive glycogen storage associated with the clinical and other features of type I glycogenosis in whom the enzyme defect is not demonstrable in vitro (4 25) This paper reports the results of detailed studies in such a case

THE PATIENT

The patient was the first child of healthy unrelated parents and had a healthy younger sister Gestation and delivery were normal (birth weight 2 2 kg) and he had presented at 11 weeks with an irreducible right inguinal hernia and an enlarged liver (lower edge palpable 8 cm below the right costal margin blood urea 9 3 mmol/l plasma bicarbonate 5 mmol/l blood glucose 2 8 mmol/l) He had been seen again at 5 months because of an attack of diarrhoea which lasted 5 weeks Growth retardation liver enlargement into the right iliac fossa and a poor response to oral glucose with a flat glucose tolerance curve were recorded at one year The enlarged liver accumulated radioactive colloid (metastable technetium) poorly and this was associated with increased uptake by the normal size spleen Intravenous urography showed normal size kidneys

A liver biopsy at 14 months showed abnormal storage of glycogen but no enzymological evidence to support a diagnosis of either Types I III or III glycogenosis (see Results) Random specimens of urine consistently con

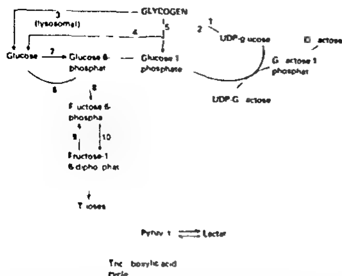


Fig 1 Simplified scheme showing the enzymes which are involved in the biosynthesis and degradation of glycogen The asterisks indicate the enzymes which are deficient in the known glycogen storage diseases *1*=UDPG glycogen glycosyl transferase (glycogen synthase) (EC2 4 1 11) *2*=1 4-α glucan branching enzyme (EC2 4 1 18) *3*=α glucosidase (acid maltase) (EC3 2 1 20) *4*=phosphorylase (EC2 4 1 1) *5*=amylo-1 6-glucosidase and transferase (debranching enzyme) (EC3 2 1 33) and 4-α glucanotrans ferase (EC2 4 1 25) *6*=glucose-6-phosphatase (EC3 1 3 9) *7*=glucokinase (EC2 7 1 2) *8*=glucose phosphate isomerase (EC5 3 1 9) *9*=fructose 1 6-diphosphatase (EC3 1 3 11) *10*=phosphofructokinase (EC2 7 1 4)

tained large amounts of lactic and 2-oxoglutaric acids Abnormal 2 oxoglutaric aciduria had not been previously reported in any of the glycogenoses and the patient was therefore admitted to The Clinical Research Centre when he was 23 months old At that time he was a stunted alert child with a florid face puffy cheeks and the doll like facies suggestive of type I glycogenosis His height (69 cm) and weight (8 6 kg) were both below the 3rd percentile and his bone age was 9 months The only other physical abnormality was gross abdominal distension due to hepatomegaly There were no hypoglycaemic symp toms and no evidence of psychomotor retardation except in so far as the abdominal distension reduced his overall mobility The results of the routine haematological exami nations on admission were as follows haemoglobin 11 5 g/dl packed cell volume 35 1% mean corpuscular vol ume 89 fl mean corpuscular haemoglobin 29 2 pg mean corpuscular haemoglobin concentration 32 7 pg total white cell count 12 200/μl with 28% neutrophils 69% lymphocytes 1% monocytes 2% basophils platelet count 257 000/μl prothrombin time kaolin cephalin time and thrombin time normal Urine centrifuge deposit was normal and sterile on culture throat and rectal swabs showed normal flora only The results of the clinical chemistry investigations are summarised in *Table 1* The child was grossly and consistently hypoglycaemic after an overnight fast and before feeds he was also markedly hyperlactic acidaemic

The abnormal urinary organic acid excretion patte was confirmed using timed urine collections (see Results The plasma and urine concentrations of free amino aci were normal except for slightly increased excretions alanine and glycine (P Purkiss) The urine did not conta detectable amounts of glucose fructose or galactose T findings during glucagon oral glucose and oral galacto tolerance tests were compatible with a diagnosis glycogenosis type I and at variance with the results of t in vitro enzyme assays (see Results) The patient w discharged on a regime of frequent feeds with a hi protein content

He was readmitted for reassessment at 36 months ha ing remained well and gained weight The results of t routine biochemical and haematological investigatio were normal Blood glucose levels were satisfactory e cept for concentrations of 0 5 mmol/l and <0 1 mmc immediately before breakfast and immediately bef re t respectively The hyperlactic acidaemia (4–9 mmol persisted throughout the 24 hours He was discharged 3 hourly feeds with milk and Marvel' dried low fat ski med milk (Cadbury s Ltd) supplements during the nig An episode of acute gastroenteritis during the admissi produced profound hypoglycaemia hyperlactic acidaer and circulatory collapse from which however he made good recovery The patient was seen again at 39 mont and he remained well except that he had had an attack pneumonia two months previously He was also b

Table 1 *Clinical chemistry*

	Observed result	Normal range (child)
Blood urea	4 4 mmol/l	2 5–6 3 mmol/l
Serum sodium	135 mmol/l	136–147 mmol/
Serum potassium	4 2 mmol/l	3 8–5 2 mmol/l
Plasma bicarbonate	12 mmol/l	0–26 mmol/l
Total serum protein	88 g/l	60–80 g/l
Serum albumin	49 g/l	35–48 g/l
Serum globulin	39 g/l	25–37 g/l
Serum α_1 globulin	2 g/l	1– 4 g/l
Serum α_2 globulin	17 g/l	5–10 g/l
Serum β globulin	16 g/l	6–12 g/l
Serum γ globulin	4 g/l	7–17 g/l
Plasma thyroxine	[illegible] mmol/l	60–150 mmol/l
T_3 uptake	114%	95–118%
Blood ammonia	19 μmol/l	13–34 μmol/l
5 nucleotidase	26 6 IU/l	10–17 IU/l
Aspartate trans aminase	168 IU/l	<40 IU/l
Alkaline phosphatase	15 5 K A Units	10–20 K A Units
Serum cholesterol	7 7 mmol/l	4 0–6 0 mmol/l
Serum triglycerides	12 5 g/l	<1 8 g/l
Serum lipoprotein pattern	Grossly abnormal	–
Serum L lipoprotein	2 5 g/l	0–0 28 g/l
Serum M lipoprotein	13 1 g/l	[illegible] 1–2 4 g/l
Serum S lipoprotein	3 68 g/l	2 3–5 5 g/l
Serum uric acid	279 μmol/l	100–350 μmol/l
Blood sugar after overnight fast	<0 3 mmol/l	3 5–6 0 mmol/l

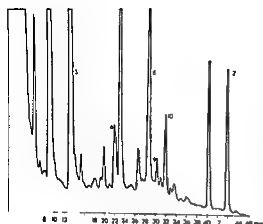


Fig 2 Gas chromatogram of the urinary organic acids as trimethylsilyl and trimethylsilyl-ethoxime derivatives separated on 10% OV 101 on HP Chromosorb W 80–100 mesh by temperature programming from 110°C to 285 C [redacted] 4 C/min with a 5 min initial isothermal delay Peak identifications are *1* lactate *2* sulphate *3* phosphate *4* glycerate (+) *5* ?-deoxytetronate *6* tetronates *7* 2-oxoglutarate *8* citrate *9* 4-hydroxyphenyllactate *10* undecandioate (internal standard) *11* *n* tetracosane (internal standard) *12* *n* hexacosane (internal standard)

chemically normal except for blood sugars in the hypoglycaemic range before meals and a fasting blood lactate of 6 4 mmol/l Blood from the patient and his mother was examined at this time for glycogen and the leucocytes for amylo-1 6-glucosidase activity The levels in the patient were 38 μg/ml and 0 54 mmol/mg protein/min respectively and those [redacted] his mother were [redacted] μg/ml and 0 54 mmol/mg protein/min respectively levels of glycogen were low (normal 40–700 μg/ml) and the amylo-1 6-glucosidase activity was normal This was further evidence against a diagnosis of glycogenosis Type III The patient s blood contained many spherocytes suggesting that he might be developing haemolytic anaemia

The episodes of acidosis hypoglycaemia and collapse following m ld infections became more frequent and more severe and he was admitted to a hospital near his home at 30 months Intravenous bicarbonate and glucose produced symptomatic improvement but he remained acidotic and d ed when he was 31 months old Permission for an autopsy was refused and further biochemical studies on the liver were not possible

SPECIAL ANALYTICAL METHODS

Tissue enzyme assays and glycogen determination The methods used are described by Ryman (18) and Eagle et al (7)

Urinary non amino organic acids were measured using gas liquid chromatography (5 11) Peak identifications were confirmed by on line mass spectrometry (Dr A M Lawson)

Plasma and urinary amino acids were measured with a Technicon Model TSM Amino Acid Analyser (P Purkiss)

Urinary sugars The gas chromatographic/mass spectrometric method described by Lawson [redacted] (14) was used

RESULTS

Liver glycogen content and enzyme activities

Table 2 shows the glycogen content and the activities of the following enzymes in the liver biopsy taken when the child was 14 months old glucose 6-phosphatase (EC3 1 3 9) amylo-1 6-glucosidase (EC3 2 1 33) liver glycogen phosphorylase (EC2 4 1 1) and fructose 1 6-diphosphatase (EC3 1 3 11)

Urinary non amino organic acids

In addition to the consistently abnormal pattern observed on random urine samples collected between the ages of 14 and 23 months the following results were obtained on two consecutive timed 24 hour collections The urine volumes and creatinine levels were low (100 and 136 ml and 0 48 and 0 51 mmol/24 hours respectively) Lactic acid levels were grossly elevated at 0 52 mmol/24 hours on each occasion (0 86 to 0 89 μg per μg creatinine respectively lactic acid is not normally present in children s urine as determined by these methods) The 2-oxoglutaric acid excretions were 0 22 and 0 28 mmol/24 hours

Table 2 *Glycogen concentration and enzyme activity in the liver tissue*[a]

	Patient	Normal range[b]
Glycogen (mg/100 mg)	13 0	1–4
Glucose-6-phosphatase (μmol Pi released/min/g)	5 0	3–9
Amylo-1 6-glucosidase (μmol glucose released from α glucosyl Schardinger dextrin/min/g)	1 2	0 7–1 1
Phosphorylase (μmol Pi released/min/g)	{7 8 / 18 8	20
Fructose 1 6-diphosphatase (μmol NADPH generated/min/g)	7 7	3–5

[a] Related to unit wet weight of the tissue
[b] Established in the authors (B E R s) laboratory (Methods as described by Ryman (18))
Separate biopsies

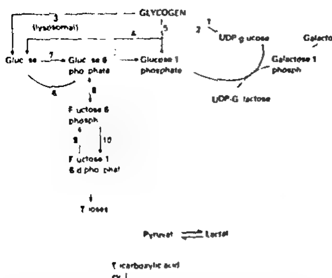


Fig 1 Simplified scheme showing the enzymes which are involved in the biosynthesis and degradation of glycogen The asterisks indicate the enzymes which are deficient in the known glycogen storage diseases *1*=UDPG glycogen glycosyl transferase (glycogen synthase) (EC2 4 1 11) *2*=1 4-α glucan branching enzyme (EC2 4 1 18) *3*=α glucosidase (acid maltase) (EC3 2 1 20) *4*=phosphorylase (EC2 4 1 1) *5*=amylo-1 6-glucosidase and transferase (debranching enzyme) (EC3 2 1 33) and 4-α glucanotrans ferase (EC2 4 1 25) *6*=glucose-6-phosphatase (EC3 1 3 9) *7*=glucokinase (EC2 7 1 2) *8*=glucose phosphate isomerase (EC5 3 1 9) *9*=fructose 1 6-diphosphatase (EC3 1 3 11) *10*=phosphofructokinase (EC2 7 1 4)

tained large amounts of lactic and 2 oxoglutaric acids Abnormal 2 oxoglutaric aciduria had not been previously reported in any of the glycogenoses and the patient was therefore admitted to The Clinical Research Centre when he was 23 months old At that time he was a stunted alert child with a florid face puffy cheeks and the doll like facies suggestive of type I glycogenosis His height (69 cm) and weight (8 6 kg) were both below the 3rd percentile and his bone age was 9 months The only other physical abnormality was gross abdominal distension due to hepatomegaly There were no hypoglycaemic symptoms and no evidence of psychomotor retardation except in so far as the abdominal distension reduced his overall mobility The results of the routine haematological examinations on admission were as follows haemoglobin 11 5 g/dl packed cell volume 35 1% mean corpuscular volume 89 fl mean corpuscular haemoglobin 29 2 pg mean corpuscular haemoglobin concentration 32 7 pg total white cell count 12200/μl with 28% neutrophils 69% lymphocytes 1% monocytes 2% basophils platelet count 257000/μl prothrombin time kaolin cephalin time and thrombin time normal Urine centrifuge deposit was normal and sterile on culture throat and rectal swabs showed normal flora only The results of the clinical chemistry investigations are summarised in Table 1 The child was grossly and consistently hypoglycaemic after an overnight fast and before feeds he was also markedly hyperlactic acidaemic

The abnormal urinary organic acid excretion p… was confirmed using timed urine collections (see Resul… The plasma and urine concentrations of free amino aci… were normal except for slightly increased excretions… alanine and glycine (P Purkiss) The urine did not con… detectable amounts of glucose fructose or galactose T… findings during glucagon oral glucose and oral galact… tolerance tests were compatible with a diagnosis… glycogenosis type I and [redacted] variance with the results of… in vitro enzyme assays (see Results) The patient… discharged on a regime of frequent feeds with a h… protein content

He was readmitted for reassessment at 26 months… ing remained well and gained weight The results of… routine biochemical and haematological investigat… were normal Blood glucose levels were satisfactory… cept for concentrations of 0 5 mmol/l and <0 1 mm… immediately before breakfast and immediately befor… respectively The hyperlactic acidaemia (4–9 mm… persisted throughout the 24 hours He was discharge… 3 hourly feeds with milk and Marvel dried low fat… med milk (Cadbury s Ltd) supplements during the n… An episode of acute gastroenteritis during the admi… produced profound hypoglycaemia hyperlacti acida… and circulatory collapse from which however he m… good recovery The patient was seen again at 29 m… and he remained well except that he had had an atta… pneumonia two months previously He was also

Table 1 *Clinical chemistry*

	Observed result	Normal rang (child)
Blood urea	4 4 mmol/l	2 5–6 3 mm
Serum sodium	135 mmol/l	136–147 mn
Serum potassium	4 2 mmol/l	3 8–5 2 mm
Plasma bicarbonate	12 mmol/l	20–26 mmo
Total serum protein	88 g/l	60–80 g/l
Serum albumin	49 g/l	35–48 g/l
Serum globulin	39 g/l	25–32 g/l
Serum α_1 globulin	2 g/l	1– 4 g/l
Serum α_2 globulin	17 g/l	5–10 g/l
Serum β globulin	16 g/l	6–12 g/l
Serum γ globulin	4 g/l	7–17 g/l
Plasma thyroxine	98 mmol/l	60–150 mmol/l
T_3 uptake	114%	95–118%
Blood ammonia	19 μmol/l	13–34 μmol/l
5 nucleotidase	26 6 IU/l	10–17 IU/l
Aspartate trans aminase	168 IU/l	<40 IU/l
Alkaline phosphatase	15 5 K A Units	10–20 K A Units
Serum cholesterol	7 7 mmol/l	4 0–6 0 mmol/l
Serum triglycerides	12 5 g/l	<1 8 g/l
Serum lipoprotein pattern	Grossly abnormal	–
Serum L lipoprotein	2 5 g/l	0–0 28 g/l
Serum M lipoprotein	13 1 g/l	0 1–2 4 g/l
Serum S lipoprotein	3 [redacted] g/l	2 3–5 5 g/l
Serum uric acid	279 μmol/l	100–350 μmol/l
Blood sugar after overnight fast	<0 3 mmol/l	3 5–6 [redacted] mmol/l

Glucagon glucose and galactose tolerance tests

A glucagon test (1 mg glucagon administered intramuscularly 3 hours after a meal) and oral glucose (2 g/kg body weight) and oral galactose (2 g/kg body weight) tolerance tests were made The concentrations of glucose and lactate were measured by standard enzymatic methods and the results are presented in Fig 3

During the glucagon test the blood glucose levels throughout the tests fell and lactate levels rose to a peak between 30 and 45 minutes after glucagon was given The administration of glucose caused the expected rapid rise in blood glucose accompanied by a slow fall of blood lactate Galactose produced a fall in blood glucose and a rise in blood lactate levels as shown in Fig 3

DISCUSSION

The presence of severe fasting hypoglycaemia with gross hyperlactic acidaemia and accumulation of glycogen in the liver is compatible with a diagnosis of type I glycogenosis Fructose 1 6-diphosphatase deficiency can also resemble type I glycogenosis (7) However the glucose 6-phosphatase and fructose 1 6-diphosphatase activities were normal in vitro as were the other hepatic enzymes studied The patient therefore falls into the at present unclassified group of cases with an hepatic glycogen storage disease but no identifiable biochemical defect

The properties of glucose 6-phosphatase were extensively reviewed by Ryman & Whelan (19) it is a membrane bound enzyme which catalyses several reactions other than the hydrolysis of glucose 6-phosphate The phosphate liberated from glucose 6-phosphate is normally determined in the assay used for biopsy work and since glucose 6-phosphatase is readily inactivated at pH 5 0 while other phosphatases which also hydrolyse the glucose 6-phosphate are not it is essential that suitable controls are carried out as described by Hers (12) Greater stability of the homogenate in the assay is achieved by preparing the biopsy tissue in sucrose EDTA as recommended by Ricketts (16) All the precautions mentioned above were used in assessing the glucose 6-phosphatase activity in this child

The oral administration of glucose produced a rapid rise in blood glucose to a peak with a corresponding slow fall in blood lactate levels indicating that glucose absorption was normal and that in the absence of an increase in blood lactate glucokinase activity and glycogen synthesis are probably normal When glucagon was administered blood glucose levels actually fell but the blood lactate levels rose to a peak indicating normal activity of liver phosphorylase but absent glucose 6-phosphatase activity in vivo Formation of lactate from glycogen indicates a normal activity of the other intermediary enzyme systems These results are confirmed by the oral galactose tolerance test with its associated decrease in blood glucose and increase in blood lactate to a high plateau level

As in the typical cases of type I glycogenosis this patient showed chronic fasting hypoglycaemia and hyperlactic acidaemia without any symptoms of acidosis hypoglycaemic brain damage or retarded mental development It has been suggested that under these conditions lactate provides an important source of metabolic energy This is in accord with the evidence presented recently for an active dynamic pool of lactate in type I glycogenosis generated by a recycling process between glycogen and lactate (20)

The occurrence of large amounts of 2 oxoglutarate in the urine as was observed in this patient and subsequently in the urine of a patient with enzymologically proven type I glycogenesis has not been reported by other workers in any of the glycogenoses Examination of urine from patients with glycogenoses types II and III by the present methods showed only minor changes in the organic acid excretion pattern The urinary 2-oxoglutarate excretion is increased in fructose 1 6-diphosphatase deficiency (21)

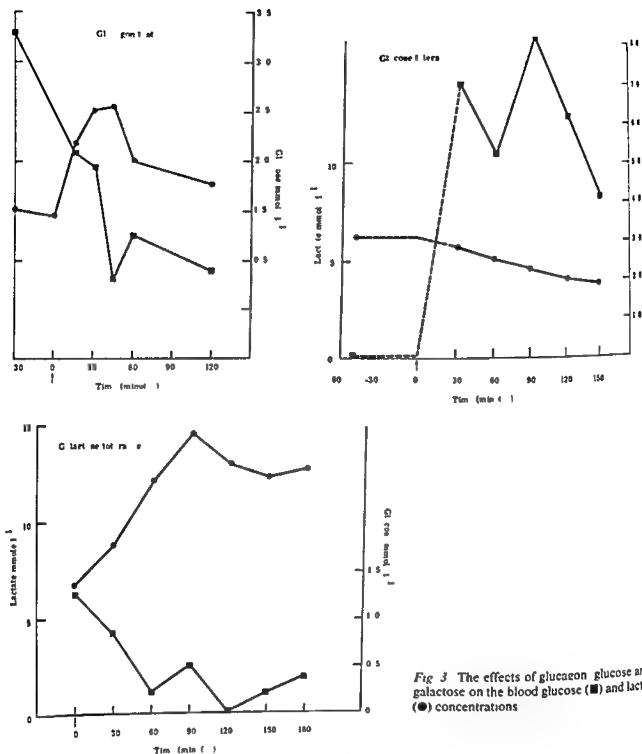


Fig 3 The effects of glucagon glucose ar galactose on the blood glucose (■) and lact (●) concentrations

(0 56 and 0 71 μg/μg creatinine respectively the normal levels are 0 032 (±S D 0 014 n=20) μg/μg creatinine in children aged 1 5 years to 5 years as determined by the present methods) The other urinary organic acids were normal A typical chromatogram of the urinary organic acids from this patient is shown in Fig 2 A similar urinary organic acid excretion pattern and levels have been observed in one patient with enzymologically proven glycogenosis Type I in whom lactic acid excretion was 0 19 μg/μg creatinine and 2 oxoglutaric acid excretion was 0 51 μg/μg creatinine Peak identifications were confirmed by on line mass spectrometry (Dr A M Lawson)

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and in pyruvate carboxylase deficiency (2) in both of which gluconeogenosis is disordered as well as in pyruvate decarboxylase deficiency (6 8) There is pyruvic aciduria as well as lactic aciduria and hyperlactic acidaemia in these three disorders Pyruvic aciduria was never observed in the present case suggesting that although pyruvate production was excessive its further metabolism by metabolic pathways other than reduction to lactate was unimpaired and the pyruvate pool was not expanded beyond the capacity of the available lactate dehydrogenase to catalyse its reduction A metabolic block on one of the pathways of pyruvate utilisation or catabolism presumably leads to a greater expansion of the pyruvate pool than a metabolic lesion which causes excessive pyruvate production as part of the recycling between glycogen and lactate described by Sadeghi Nejad et al (20) Under these circumstances the avidity of lactate dehydrogenase for the pyruvate substrate must be greater than that of the enzymes which catalyse its conversion to acetyl CoA ox aloacetate L malate or hydroxypyruvate

The reason for the excessive excretion of 2 oxoglutarate is obscure It is of interest that the 2 oxoglutarate dehydrogenase (EC1 2 4 2) enzyme complex and the pyruvate dehydrogenase (EC1 2 4 1) complex both include lipoate and thiamine pyrophosphate and that pyruvate decarboxylase (EC4 1 1 1) also requires thiamine pyrophosphate It is possible that the metabolism of large amounts of pyruvate to acetyl CoA by the reactions which are catalysed by these enzymes causes a conditioned cofactor deficiency (e g a reduced supply of oxidised lipoyl residues) which impairs the activity of 2 oxoglutarate dehydrogenase and causes 2 oxoglutarate to accumulate with an overall slowing of the tricarboxylic acid cycle The increased triglyceride and cholesterol levels could then be explained by increased metabolic turnover of acetyl CoA in the direction of lipid synthesis rather than incorporation into the tricarboxylic acid cycle via citrate

The present patient shows a discrepan between glucose 6-phosphatase activity a judged on the basis of in vivo studies and an i vitro assay A few similar cases have been re ported previously (1 3, 8 10 24 25) a some authors refer to this as type I glycogenosis (15 22 23) Several possible e planations of this may be advanced Firstl that the structural change in the enzyme pr tein reduces its affinity for the substra thereby reducing the catalytic activity in vi but not under the conditions of saturating su strate concentration which are used for t assay in vitro Secondly, the enzyme may subject to regulatory factors possibly alloste in type which are disordered in vivo but no vitro However there is little informat about the factors which regulate the activit of normal glucose 6 phosphatase The enzym is a lipoprotein which is strongly bound to th endoplasmic reticulum It is possible that th change in enzyme structure impairs its norma binding to the endoplasmic reticulum an orientation within the cell although the cata lytic site is in fact intact Alternatively th change could be in the structure of the endo plasmic reticulum to which the enzyme is a tached Either of these changes could make an ineffective catalyst in vivo but leave i properties as demonstrated under artificial i vitro conditions intact

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PAPAVERINE IN THE PROPHYLAXIS OF MIGRAINE AND OTHER VASCULAR HEADACHE IN CHILDREN

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ABSTRACT Sillanpaa M and Koponen M (Departments of Paediatrics University of Turku Turku and Central Hospital of Tampere Tampere Finland) Papaverine in the prophylaxis of migraine and other vascular headaches in children Acta Paediatr Scand 67 209 1978.—A daily dose of papaverine (5–10 mg per kg body weight) was administered on a double blind basis for two months to 42 successive child patients suffering from migraine or other vascular headache attacks not less than twice a month Thirty-seven patients received the drug for the whole period of two months and 31 of these could be followed for a period of one to 10 months (mean 4 5 months) after the cessation of the treatment Nineteen received papaverine and 18 placebo Six out of 19 papaverine patients and none of 18 placebo patients were completely free from attacks during the treatment Altogether 11 patients in the papaverine group and 5 in the placebo group experienced a 75–100% reduction in attack frequency which was accompanied by lower intensity and shorter duration of attacks The results were best in cases with classical migraine An excellent or good drug effect was experienced by 58% of papaverine patients and 17% of placebo patients

KEY WORDS Migraine headache prophylaxis papaverine children

One of the common features of childhood migraine is the high frequency of attacks often accompanied by prolonged vomiting Consequently attempts at drug prophylaxis may be considered to be well motivated We still lack an effective well tolerated drug for childhood migraine which is safe even in long term prophylactic use Papaverine a well known vasoactive agent has been shown to be effective in adult migraine (6) We carried out a double blind study to determine its effect in children with migraine or other vascular headache

PATIENTS AND METHODS

The patient series consisted of 42 successive children admitted for migraine or other vascular headache to the Paediatric Outpatient Department in Turku or Tampere from November 1 1975 to September 30 1976 Migraine was defined by the criteria of Vahlquist (8) i e paroxysmal headache separated by headache free intervals and associated with at least two of the following four nausea (or vomiting or both) unilateral pain visual aura and positive family history If only one item was present the patient was placed in the non migrainous vascular pain group Additional criteria for inclusion of patients in the latter group were throbbing pain and photosensitivity Cases with less than two well-defined attacks of headache a month and those with raised intracranial pressure were excluded from the study

The cases included were examined and followed personally by the present authors All the routine clinical physical and laboratory examinations were performed to exclude intracranial expansion intra and paracranial infections etc The patients were seen or contacted by phone one and two months from the beginning of drug therapy respectively In addition the patients and their mothers were interviewed at least once at various time intervals after the end of the treatment

The patients received papaverine (Papaverin® Star¹) 5–10 mg per kg body weight divided into two or three daily doses for two months The treatment was usually started with a daily dose of 5 mg per kg and was doubled a month later Thirty-one patients could be followed clini

The material and financial aid from STAR Pharmaceutical Co are gratefully acknowledged

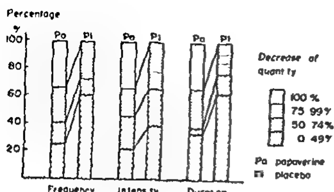


Fig 1 Decrease of frequency intensity and duration of headache attacks during papaverine and placebo treatment

Table 1 Classification of different headache types in the papaverine and placebo groups

Type of headache	Papaverine group (No of patients)	Placebo group (No of patients)
Classic migraine	5 (26%)	5 (28%
Common migraine	12 (63%)	10 (55%
Other vascular	2 (11%)	3 (17%
Total	19 (100%)	18 (100

cally for one to 10 months (mean 4 5 months) Six further patients were under control up to the end of the drug therapy During the study five patients were excluded three due to irregular administration of the drug and two because of side effects One of these experienced night restlessness and another stomach ache and nausea Both were later proved to have received papaverine

The final series thus comprised 37 children 14 boys and 23 girls The study was double blinded and when the code was opened 19 had received papaverine and 18 placebo

The age of the patients at the onset of the therapy varied from six to 15 years (mean 9 5 and medium 10 years resp)

A follow up period after cessation of treatment was possible in 31 cases (15 with papaverine and 16 with placebo) It varied from one to 10 months (mean 4 5 months)

In statistical analyses Chi Square Test and Fischer s Fourfold Table Test were applied

RESULTS

Table 1 shows the type of headache in the papaverine and placebo groups No significant differences existed between the groups Neither was there any significant difference between the two groups concerning the following single items (mean values of the total series in brackets) age at onset of headache (6 5 years) and onset of treatment (9 5 years) occurrence of nausea (90%) migraine in family (84%) unilateral pain (53%) and visual aura (27%) during attacks frequency (4 3 per months) or duration (5 5 hours) of attacks The intensity of attacks was also characterized equally by the patients in the two groups

The decrease of frequency intensity and duration of headache attacks during drug treatment is presented in Fig 1 Six out of 19 papaverine patients and none of 18 placebo patients were completely free from attacks during treatment The difference is statistically highly significant ($p<0.001$) Altogether 11 patients in the papaverine group and five in the placebo group experienced a 75–100% reduction in attack frequency Five papaverine patients and nine placebo patients had less than 50% or reduction in attack frequency Reduction of frequency was accompanied by lower intensity and shorter duration of attacks in all but patient in the two groups

Fig 2 shows the decrease of frequency intensity and duration of headache attacks after drug treatment in the mean follow up period 4 5 months compared to those before and during therapy The treatment result was highly significantly ($p<0.001$) more effective in papaverine than in placebo patients

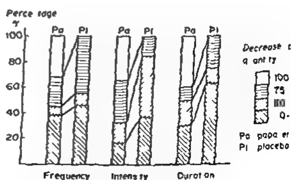


Fig 2 Decrease of frequency intensity and duration of headache attacks after papaverine and placebo treatment

Table 2 *Effect of treatment on the frequency of headache attacks of different types in papaverine and placebo groups*

Type of attack	Decrease of frequency 100–75%	74–0%	Total
Papaverine group			
Classic migraine	4 (80%)	1 (20%)	5 (100%)
Common migraine	6 (50%)	6 (50%)	12 (100%)
Other vascular	1 (50%)	1 (50%)	2 (100%)
Placebo group			
Classic migraine	3 (60%)	2 (40%)	5 (100%)
Common migraine	2 (20%)	8 (80%)	10 (100%)
Other vascular		3 (100%)	3 (100%)

One patient with absence of attacks during papaverine treatment experienced a recurrence of attacks two months later. A doubling of the daily dosage of 5 mg per kg was effective in preventing a recurrence. Two other papaverine patients, free from attacks during therapy, again developed mild attacks two to four weeks after the treatment had been stopped. One further patient with partial relief during the papaverine period experienced more intensified attacks after treatment.

In the placebo group, two patients complained of worsening attacks after treatment and another patient felt better after discontinuation of therapy than during it, although no actual side effects were complained of during therapy.

Table 2 shows that papaverine is proportionally most effective in classical migraine. The difference is statistically almost significant ($p<0.05$). The placebo effect does not seem to be influenced by the headache type.

The personal opinion of the patients and their guardians on the effect of the treatment (Table 3) disclosed that four out of five papaverine patients had experienced an excellent, good or fair effect while the same was true in only three out of five cases in the placebo group. The difference is even more remarkable if only cases with excellent or good effect are considered: 58% of papaverine

Table 3 *Treatment results experienced by patients*

Result	Papaverine group	Placebo group	Total
Good to excellent	11 (58%)	3 (17%)	14 (38%)
Fair	4 (21%)	8 (44%)	12 (32%)
No effect	3 (16%)	7 (39%)	[illegible] (27%)
Worse	1 (5%)	(0%)	1 (3%)
Total	19 (100%)	18 (100%)	37 (100%)

patients and 17% of those on placebo belonged to that group. The difference between papaverine and placebo groups is statistically significant ($p<0.025$).

DISCUSSION AND CONCLUSIONS

Although migraine attacks in childhood can often be treated with acetosalicylates, there are cases with a high frequency of attacks where prophylactic use of drugs is indicated for short periods. Many prophylactic antimigraine drugs such as cyproheptadine (1), methysergide (2–4), propranolol (5) and clonidine (7) have been demonstrated to be of some use even in children but their untoward effects have in many cases prevented their continuous use.

Papaverine is an old and well known drug with few side effects. To our knowledge, no previous communications exist on its effect on childhood migraine.

The present results show that papaverine is useful in the prophylaxis of migraine and other vascular headaches, regardless of the type of headache, although classical migraine is best influenced by the drug.

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SUBCLINICAL PROTEIN ENERGY MALNUTRITION IN UNDER PRIVILEGED ETHIOPIAN MOTHERS AND THEIR NEWBORN INFANTS

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From the [1]Ethiopian Nutrition Institute Addis Abeba the [2]Lideta Mother and Child Health Training and Demonstration Center Addis Abeba Ethiopia and the [3]Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Gebre Medhin M Larsson U Lindblad B S and Zetterstrom R (Ethiopian Nutrition Institute Addis Abeba Lideta Mother and Child Health Training and Demonstration Center Addis Abeba Ethiopia and Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) Subclinical protein-energy malnutrition in under privileged Ethiopian mothers and their newborn infants Acta Paediatr Scand 67 213 1978.—An increased glycine/valine ratio in the maternal and cord vein plasma was observed in under privileged women and their newborn babies in Ethiopia There was a delay in the decrease of branch-chained amino acids during the immediate neonatal period These findings indicate malnutrition in utero and are identical with those of a previous study in an under privileged group in Pakistan A reduced cord/maternal ratio of tyrosine and methionine was found in the Ethiopian material indicating placental dysfunction of unknown origin

KEY WORDS Protein-energy malnutrition mother newborn developing country amino acids plasma

It has been reported (6) that the dietary intake during pregnancy among under privileged Addis Abeba women is grossly deficient compared with the recommended level A study of birth weight for gestational age conducted in the same population group (8) indicated that the newborn infants displayed faltering intra uterine growth which is manifested at 34–35 weeks of gestation Lindblad et al (15) observed that mothers and newborn infants of a very low socio-economic group in Pakistan showed aberrations of the amino acid concentrations of plasma that were consistent with those described in postnatal protein-energy malnutrition (2 9)

The present investigation was undertaken in order to find out whether pregnant Ethiopian mothers and their newborn babies who are socio-economically similar to but ethnically different from those surveyed in Pakistan show these biochemical characteristics of malnutrition The study also aimed at examining the validity of previous observations in which the plasma glycine/valine ratio was used to diagnose nutritional deficiency during pregnancy (12)

MATERIAL AND METHODS

Clinical material

The subjects of the investigation were all recruited from a comprehensive study of the nutritional aspects of pregnancy conducted at the Ethiopian Nutrition Institute during the years 1969–1975 The groups examined are presented in Table 1 The mean birth weight of the infants was 3 110 g Five infants weighed less than 3 000 g but none were below 2 500 g

The *privileged* women (ETI A) who attended the University Department of Obstetrics and Gynaecology had completed at least secondary school education and their monthly family income was well above Eth$600 The

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7 Sillanpaa M Clonidine prophylaxis of childhood migraine and other vascular headache *Headache 17* 28 1977
8 Vahlquist B Migraine in children *Int Ann Ne* *7* 348 1955

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MATERIAL AND METHODS

Clinical material

The subjects of the investigation were all recruited from a comprehensive study of the nutritional aspects of pregnancy conducted at the Ethiopian Nutrition Institute during the years 1969–1975 The groups examined are presented in Table I The mean birth weight of the infants was 3110 g Five infants weighed less than 3000 g but none were below 2500 g

The *privileged* women (ETI A) who attended the University Department of Obstetrics and Gynaecology had completed at least secondary school education and their monthly family income was well above Eth$600 The

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Table 2 *All statistically significant differences between the venous plasma free amino acid levels of under privileged Ethiopian mothers and their newborn infants and normal Swedish levels (13 14)* (μmol/l)

	Ethiopian underprivileged			Swedish normal			Level of significance
	n	Mean	S D	*n*	Mean	S D	*p*
Maternal during delivery							
Glycine	14	215	57	10	104	35	<0 001
Glycine/valine ratio	14	1 65	0 35	10	0 87	0 27	<0 001
Cord vein during delivery							
Glycine	15	330	63	10	239	35	<0 001
Glycine/valine ratio	15	1 61	0 36	10	1 07	0 18	<0 001
Phenylalanine	15	88	20	10	67	10	<0 005
Methionine	15	33	9	10	18	6	<0 001
Cord vein/maternal ratio							
Tyrosine	14	1 74	0 56	10	2 47	0 58	<0 01
Methionine	14	1 66	0 46	9	2 36	0 72	<0 01
Newborn 4 hrs after birth							
Isoleucine	7	53	15	10	36	5	<0 005

of the glycine/valine ratio The glycine level and the glycine/valine ratio were also significantly increased in the cord vein plasma as compared with a normal Swedish material (14) (Table 2 and Fig 2) Both in the maternal and in the cord vein plasma there was a general tendency towards raised free amino acid levels

The phenylalanine and methionine concentrations in cord plasma were significantly increased but the valine and isoleucine concentrations were normal

The ratios between the cord and the maternal amino acid concentrations were generally low as compared with those found in the normal Swedish material (14) with the exception of alanine and urea The tyrosine and methionine concentrations in particular showed very low cord/maternal ratios (Table 2)

In the newborn infant *4 hours after birth* before initiation of feeding the alanine glycine and proline concentrations were higher (0 01<*p*<0 1) than those observed in a normal Swedish material (13) The branch chained amino acids especially isoleucine displayed higher levels during this period (Table 2 and Fig 3)

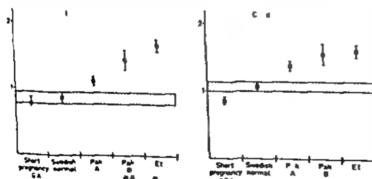


Fig 2 The glycine/valine ratio during delivery The maternal and cord plasma ratios of glycine/valine during delivery of the under privileged Ethiopian group are compared with those of appropriate for gestational age (*AGA*) Swedish infants after a short pregnancy those of normal Swedish deliveries (14) and those of two socio-economic groups (*A* = low socio-economic group and *B* = very low socio-economic group) in Pakistan (15) Mean ±S E M are indicated 0 001<*p*<0 01 *p*<0 001

Table 1 *Ethiopian mothers investigated during pregnancy with sampling from the cubital vein and during delivery with simultaneous sampling from the cubital vein of the mother and cord vein*

Group	Age (y)	Parity	Weight (kg)	Height (cm)	Income (Eth$)	Birth weight of infant (g)
During pregnancy (28th–38th week)						
Privileged ($n=5$)						
Mean	28.2	1	63.4	160.2	1 236.00	
Range	20–38		53–70	146–172	600–1 980	
Under privileged ($n=5$)						
Mean	18.2	1	58.4	151.0	68.6	
Range	15–24		54–60	142–158	50–125	
During delivery						
Under privileged ($n=15$)						
Mean	24.1	2.2			56.3	3 110
Range	17–38	1–5			8–300	2 630–3 850

Eth$1 = US$0.49

under privileged women (ETI B) and their offspring were recruited from a municipal clinic for the indigent in Addis Abeba. The majority of these women had at most a year or two of primary school education and the family income rarely exceeded Eth$100 (US$49) per month.

All the women who participated in the study were free from obvious signs of acute or chronic disease, their blood pressure was normal and there was no glucose or protein in the urine. They were recruited consecutively during a short period of time.

Examination methods

The length of gestation was determined by interviewing the women, using a specially designed local calendar.

Anthropometric measurements were performed by standard methods (10).

In the groups investigated during pregnancy (privileged and under privileged), fasting morning blood samples were drawn from the mothers on one occasion between the 28th and 38th week of gestation. In those investigated during delivery (under privileged) blood was collected simultaneously from the mother and from the cord vein immediately following delivery and also from the newborn infant 4 hours after birth before initiation of feeding. Data during delivery of the privileged group were too scarce for evaluation, due to practical difficulties in collecting samples.

The procedures used for the storage, transport and deproteinization of samples were identical with those of previous studies (16).

Ion exchange chromatography was performed on a Bio cal 200 (München) automatic amino acid analyser (19) with a lithiumbuffer system (3).

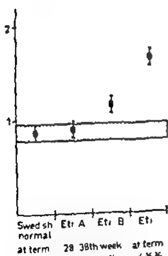


Fig. 1. The maternal glycine/valine ratio. The normal Swedish ratio during delivery at term (14) is compared with the ratios of the privileged (*ETI A*) and under privileged (*ETI B*) Ethiopian mothers during pregnancy (28–38 weeks) and with that of the underprivileged group at term (*ETI*). * $0.01<p<0.10$ ** $p<0.001$

RESULTS

The maternal glycine/valine ratio during *pregnancy* was normal in the privileged group (ETI A) as compared with Swedish levels at the time of delivery (14) and not significantly elevated ($0.01<p<0.1$) in the under privileged group (ETI B). During *delivery* in the under privileged group (ETI) the ratio was highly significantly elevated (Table 2 and Fig. 1).

During *delivery* the maternal plasma showed a significant increase of the glycine levels and

in Ethiopian newborn infants (8) The combination of an increased fetal glycine/valine ratio and higher branch chained amino acid levels neonatally speak for a combined fetal undernutrition of energy and protein The cause of the fetal malnutrition could be the altered content of essential nutrients in the maternal plasma due to dietary inadequacies However the reduced cord/maternal ratio of some essential amino acids points to a component of placental dysfunction of unknown etiology

ACKNOWLEDGEMENTS

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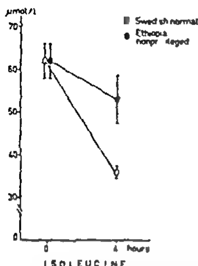


Fig 3 Isoleucine concentration in cord vein plasma during delivery and in cubital vein plasma 4 hours after birth (before the first feed) Values of under privileged Ethiopian newborns are compared with normal Swedish values (13) Mean ±S E M are indicated

DISCUSSION

The plasma levels of free amino acids increase when peripheral release from muscle tissue exceeds that of liver uptake and vice versa (4) During both experimental and clinical protein energy malnutrition in all age groups (12) the glycine level increases and the valine level decreases leading to an increase of the fasting glycine/valine ratio In fig 4 the characteristic changes in the plasma concentrations of free amino acids have been projected onto the 'glucose alanine cycle (4)

The plasma aminogram of under privileged Ethiopian mothers during delivery and their newborns conforms with that found in the socio economically similar but ethnically different group of Pakistani women (15) The pattern observed is consistent with the aberrations found both in postnatal protein as well as protein energy undernutrition (2 9 12)

The hyperaminoacidemia observed in these newborn infants agrees with that found in small for dates (SFD) babies of hypertensive women (13) The higher branch chained amino acid levels (see the isoleucine level in Fig 3) is in accordance with what is found during the early phase of acute experimental starvation where this pattern is probably secondary to the immediate drop of the insulin level seen i acute starvation (1) This finding conform with the low insulin levels and low tolerance t i v glucose and amino acids found in some newborn infants who are short and light for gestational age (17) This delay in the reduction of the branch chained amino acid levels (Fig 3) has also been observed in newborn SFD infants of mothers with hypertension where intra uterine malnutrition may be caused by placental insufficiency (13)

The changed glycine/valine ratio seen in the newborn infants cannot be due to low birth weight resulting from premature birth as the ratio of the early born is lower than normal (Fig 2) (18) This is supported by observations in the rhesus monkey where the cord glycine level increases and the valine level decreases with increasing gestational age (11)

The findings in the present survey provide evidence that malnutrition is a contributory factor of retarded growth in utero as observed

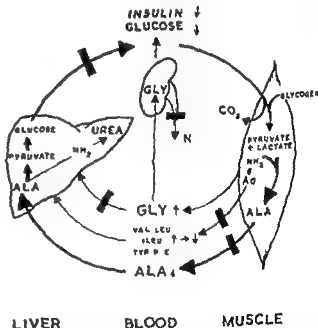


Fig 4 Postulated relations between glucose amino acid and insulin levels in starvation The characteristic changes in clinical and experimental protein energy malnutrition (12) are projected onto the glucose alanine cycle (4) whereby alanine is synthesized in muscle by transamination of glucose-derived pyruvate Alanine will accumulate in the blood if the peripheral release exceeds the rate of hepatic uptake Black bars indicate decreased function observed during starvation (5)

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RENAL FUNCTION IN ANOREXIA NERVOSA

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ABSTRACT Aperia A Broberger O and Fohlin L. (Department of Paediatrics St Goran s Hospital Stockholm Sweden) Renal function in anorexia nervosa Acta Paediatr Scand 67 219 1978.—Renal function was examined in twelve patients eight girls and four boys with anorexia nervosa (AN) ranging in age from 12 6 to 18 2 years The weight loss at the time of the study averaged 26 % Determinations were made of glomerular filtration rate (GFR) PAH clearence (C_{PAH}) and urinary concentrating capacity For references the same studies were also carried out in five healthy teenagers Both GFR and C_{PAH} were generally reduced in AN The reduction of GFR was however out of proportion to the reduction of C_{PAH} as shown by a significantly lower filtration fraction (FF) in AN Indirect evidence suggests that the low FF could be attributed to reduced water permeability of the glomerular capillary The urinary concentrating capacity following fluid deprivation was moderately depressed both before and after the administration of vasopressin The concentrating defect in AN must therefore be primary of renal origin

KEY WORDS Kidney anorexia nervosa glomerular capillaries urinary concentrating capacity malnutrition

Anorexia nervosa (AN) is commonly reported to be associated by conflicting disturbances in fluid homeostasis such as edema (3 9) and reduced urinary concentrating capacity (14) The origin of these disturbances is still un known This study was therefore undertaken to evaluate the role of the kidney in the control of fluid homeostasis in AN condition

MATERIAL AND METHODS

Eight female and four male patients suffering from anorexia nervosa were studied during the years 1975–1977 All patients conformed to the following criteria for diagnosis (chiefly from Dally (3))

1 Age at onset less than 25 years

2 Active refusal to eat with accompanying pronounced weight loss

3 No evidence of schizophrenia severe depression or organic disease

This study was supported by grants from the Swedish Medical Research Council (B77 19X 049-10B) and by grants from the Karolinska Institute

The physical characteristics of the patients are given in Table 1 All the patients except two girls and one boy are included in an enlarged study (7) The patients were treated at St Göran s Hospital for Children None of the patients showed any signs of electrolyte disturbances Blood volume per kg body weight averaged 79 ml which corresponds well with normal data The patients 24-hour excretion of aldosteron was on the lower normal level for the laboratory Only one patient (UP) was on a medication (Thioridazin chloride Mallorol®) Secondary amenorrhea was present in all postpubertal girls The patients mean weight and height were *39 6±7 4 kg and 168 5±11 3 cm* Individual weight loss from premorbid weight is given in Table 1

For reference 5 healthy subjects were examined four boys and one girl Their age varied between 14 3 and 16 5 years (mean 14 8 years) Controls mean weight and height were 53 4±7 6 kg and 168 1±9 5 cm The investigation was performed after the informed consent by all individual persons as well as by their parents and after the approval by the ethical Committee of Karolinska Institutet

All studies were performed within one week The following tests were included in the study Determination of maximal urine osmolality after fluid deprivation with and without pitressin determination of the glomerular filtration rate (GFR) by the clearance of inulin determination of renal blood flow by the clearance of PAH Serum pro-

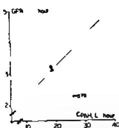


Fig 1 The relationship between PAH clearance and glomerular filtration rate

Clearance studies

GFR and renal blood flow was determined by the clearance of inulin and the clearance of PAH (C_{PAH}) with the patients fasting For this purpose a continuous infusion 0 1-0 ? ml/min of 25% Inutest® (Laevasar-Gesellschaft) and ?0% PAH (5 3 1) was given preceeded by a prime dose of 0 3 ml/kg body weight The continuous infusion was started 60 min before the first urine collection period Urine was sampled by spontaneous voiding and to ensure a constant urine flow the patients were allowed to drink water in an amount of 0 25% of the body weight every half hour In general clearance periods were 1 hour Blood samples were drawn in the middle of each clearance period from a needle in a peripheral vein Blood and urine samples were analyzed for inulin PAH urea sodium and osmolality

Sodium and potassium in serum and urine were determined by a flame photometer Osmolality was determined cryoscopically using a Knauer microosmometer Inulin was determined using the Anthron method (8) PAH was determined according to the method of Smith et al (18) Urea nitrogen serum and urine were determined by analyzing for ammonia calorimetrically before and after the action of urease (6)

RESULTS

Relevant clinical data from the patients studied are summarized in Table 1 The mean arterial blood pressure is consistently reduced in AN The reduction averages 19 mmHg The serum protein concentration is slightly lower in AN The difference averages 2 4 g/l

The effect of AN on renal hemodynamics is shown in Table 2 and Fig 1 The GFR in AN ranges from 3 04 to 5 72 1/1 73 m²/hour and is significantly lower than in the controls The average clearance of PAH is slightly lower in AN than in controls but the difference is not significant The individual variation for C_{PAH} is still larger than for GFR The relationship between C_{PAH} and GFR in AN is shown in Fig 1 The correlation coefficient 0 79 is significant ($p<0$ 01) The quotient between GFR and C_{PAH} the filtration fraction (FF) is however lower in AN than in controls The depression of the FF appears to relate well to the severity of the disease ($r=0$ 75 $p<0$ 01) It gives no correlation to the arterial blood pressure (Fig 2*a* and *b*)

Table 3 shows excretion of Na^+ and urea in relation to GFR No significant difference was found between AN and controls The serum values for Na^+ and urea are also included in the table The serum urea value was slightly but significantly higher in AN The serum Na^+ value was the same in AN as in controls

The urinary concentrating capacity is generally moderately depressed in AN (Table 4) The mean maximal urinary osmolality following 17 hours fluid deprivation and the administration of exogenous ADH was not significantly lower in AN than in controls In AN patients the maximal urinary osmolality was also determined following stimulation of endogenous ADH production by 17 hours fluid deprivation only The urinary osmolality was then slightly but not significantly lower than following exogenous ADH administration

DISCUSSION

Reduction of GFR FF and urinary concentrating capacity were the pathophysiological features in AN patients Those findings are consistent with the few previous observations that have been made in AN (17) They also agree to a certain extent with the results from the studies on renal function in patients with malnutrition (10) In malnourished children

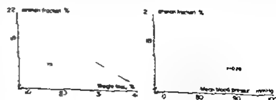


Fig 2 The relationship between weight loss (*a*) mean blood pressure (*b*) and filtration fraction

Table 1 *Relevant clinical data in patients with AN*
n s =non significant

Patients	Sex	Age (y)	Duration (y)	Weight loss (%)	Mean blood pressure (mmHg)	Serum protein (g/l)
PC	M	12 6	1 0	27	84	63
MS	M	17 0	1 0	23	74	68
UP	M	17 4	1 0	27	80	[illegible]
AN	M	18 2	1 0	23	89	79
JJ	F	12 8	0 8	35	68	72
ME	F	13 6	0 8	24	84	63
CP	F	14 2	1 3	15	81	68
AS	F	14 8	1 0	26	82	74
YN	F	14 9	0 6	15	76	68
PW	F	16 7	1 0	31	76	63
GG	F	17 0	3 5	39	72	64
KÅ	F	17 3	2 5	31	82	69
M		15 5	1 3	26 3	78 9	67 8
S D		1 9	0 8	7 0	5 8	5 1
Controls (n=5)						
M		14 8			97 0	70 2
S D		0 9			4 0	3 4
p		n s			<0 001	n s

tein was analyzed with a refractometric method Blood pressure was measured with a cuff and mean arterial blood pressure calculated as the diastolic pressure plus 40% of the pulse amplitude

Determination of maximal urine osmolality

Nine of the patients were deprived of fluid and food for 17 hours starting at 5 o clock p m Two to three urine samples were obtained by spontaneous voiding and used for determination of osmolality The fluid deprivation test was repeated but at this time an intramuscular injection of vasopressin (Pitressin tannate in oil®) in a dose of 0 5 pressor units/6 kg of body weight was given [illegible] the start of the fluid deprivation Two to three urine samples obtained by spontaneous voiding were obtained after 17 hours and analyzed for urea and osmolality

Table 2 *Renal hemodynamics in patients with AN*
n s =non significant

Patients	GFR		C_{PAH}		FF (%)
	l/hour	l/hour 1 73 m² b s	l/hour	l/hour 1 73 m² b s	
PC	3 46	4 94	25 17	35 99	13 7
MS	4 09	4 30	29 20	30 61	14 0
UP	4 28	4 54	32 83	34 85	13 0
AN	4 63	4 99	27 47	28 63	16 9
JJ	3 16	4 52	23 11	33 04	13 7
ME	3 16	3 56	18 76	21 21	16 8
CP	4 16	5 72	20 27	28 74	20 5
AS	3 13	3 64	19 22	22 33	16 4
YN	3 03	4 30	15 92	22 58	19 0
PW	2 30	3 04	17 55	22 83	13 1
GG	2 92	4 01	19 17	26 32	15 2
KÅ	4 20	4 93	30 71	37 15	13 7
M	3 54	4 37	23 28	28 69	15 5
S D	0 71	0 74	5 66	5 71	2 4
Controls (n=5)					
M	5 77	6 20	26 36	29 26	21 9
S D	0 89	0 56	1 45	2 13	2 8
p	<0 001	<0 001	n s	n s	<0 001

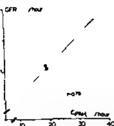


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Clearance studies

GFR and renal blood flow was determined by the clearance of inulin and the clearance of PAH (C_{PAH}) with the patients fasting For this purpose a continuous infusion 0 1-0 . ml/min of .5% Inutest® (Laevasar-Gesellschaft) and 20% PAH (5 3 1) was given preceeded by a prime dose of 0 3 ml/kg body weight The continuous infusion was started 60 min before the first urine collection period Urine was sampled by spontaneous voiding and to ensure a constant urine flow the patients were allowed to drink water in an amount of 0 25% of the body weight every half hour In general clearance periods were 1 hour Blood samples were drawn in the middle of each clearance period from a needle in a peripheral vein Blood and urine samples were analyzed for inulin PAH urea sodium and osmolality

Sodium and potassium in serum and urine were determined by a flame photometer Osmolality was determined cryoscopically using a Knauer microosmometer Inulin was determined using the Anthron method (8) PAH was determined according to the method of Smith et al (18) Urea nitrogen serum and urine were determined by analyzing for ammonia calorimetrically before and after the action of urease (6)

RESULTS

Relevant clinical data from the patients studied are summarized in Table 1 The mean arterial blood pressure is consistently reduced in AN The reduction averages 19 mmHg The serum protein concentration is slightly lower in AN The difference averages 2 4 g/l

The effect of AN on renal hemodynamics is shown in Table 2 and Fig 1 The GFR in AN ranges from 3 04 to 5 72 l/1 73 m²/hour and is significantly lower than in the controls The average clearance of PAH is slightly lower in AN than in controls but the difference is not significant The individual variation for C_{PAH} is still larger than for GFR The relationship between C_{PAH} and GFR in AN is shown in Fig 1 The correlation coefficient 0 79 is significant ($p<0\ 01$) The quotient between GFR and C_{PAH} the filtration fraction (FF) is however lower in AN than in controls The depression of the FF appears to relate well to the severity of the disease ($r=0\ 75$ $p<0\ 01$) It gives no correlation to the arterial blood pressure (Fig 2*a* and *b*)

Table 3 shows excretion of Na^+ and urea in relation to GFR No significant difference was found between AN and controls The serum values for Na^+ and urea are also included in the table The serum urea value was slightly but significantly higher in AN The serum Na^+ value was the same in AN as in controls

The urinary concentrating capacity is generally moderately depressed in AN (Table 4) The mean maximal urinary osmolality following 17 hours fluid deprivation and the administration of exogenous ADH was not significantly lower in AN than in controls In AN patients the maximal urinary osmolality was also determined following stimulation of endogenous ADH production by 17 hours fluid deprivation only The urinary osmolality was then slightly but not significantly lower than following exogenous ADH administration

DISCUSSION

Reduction of GFR FF and urinary concentrating capacity were the pathophysiological features in AN patients Those findings are consistent with the few previous observations that have been made in AN (17) They also agree to a certain extent with the results from the studies on renal function in patients with malnutrition (10) In malnourished children

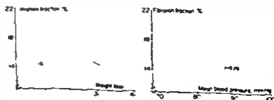


Fig 2 The relationship between weight loss (*a*) mean blood pressure (*b*) and filtration fraction

Table 1 *Relevant clinical data in patients with AN*
n s =non significant

Patients	Sex	Age (y)	Duration (y)	Weight loss (%)	Mean blood pressure (mmHg)	Serum protein (g/l)
PC	M	12 6	1 0	27	84	63
MS	M	17 0	1 0	23	74	68
UP	M	17 4	1 0	27	80	63
AN	M	18 2	1 0	23	89	79
JJ	F	12 8	0 8	35	68	77
ME	F	13 6	0 8	24	84	63
CP	F	14 2	1 3	15	81	68
AS	F	14 8	1 0	26	[illegible]	74
YN	F	14 9	0 6	15	76	68
PW	F	16 7	1 0	31	76	63
GG	F	17 0	3 5	38	72	64
KÅ	F	17 3	2 5	31	[illegible]	69
M		15 5	1 3	26 3	78 9	67 8
S D		1 9	0 8	7 0	5 8	5 1
Controls (n=5)						
M		14 8			97 0	70 2
S D		0 9			4 0	3 4
p		n s			<0 001	n s

tein was analyzed with a refractometric method Blood pressure was measured with a cuff and mean arterial blood pressure calculated as the diastolic pressure plus 40% of the pulse amplitude

Determination of maximal urine osmolality

Nine of the patients were deprived of fluid and food for 17 hours starting at 5 o clock p m Two to three urine samples were obtained by spontaneous voiding and used for determination of osmolality The fluid deprivation test was repeated but at this time an intramuscular injection of vasopressin (Pitressin tannate in oil®) in a dose of 0 5 pressor units/6 kg of body weight was given at the start of the fluid deprivation Two to three urine samples obtained by spontaneous voiding were obtained after 17 hours and analyzed for urea and osmolality

Table 2 *Renal hemodynamics in patients with AN*
n s =non significant

Patients	GFR l/hour	GFR l/hour 1 73 m² b s	C_{PAH} l/hour	C_{PAH} l/hour 1 73 m² b s	FF (%)
PC	3 46	4 94	25 17	35 99	13 7
MS	4 09	4 30	29 20	30 61	14 0
UP	4 28	4 54	32 83	34 85	13 0
AN	4 63	4 99	27 47	28 63	16 9
JJ	3 16	4 52	23 11	33 04	13 7
ME	3 16	3 56	18 76	21 21	16 8
CP	4 16	5 72	20 27	28 74	20 5
AS	3 13	3 64	19 22	22 33	16 4
YN	3 03	4 30	15 92	22 58	19 0
PW	2 30	3 04	17 55	22 83	13 1
GG	2 92	4 01	19 17	26 32	15 2
KÅ	4 70	4 93	30 71	37 15	13 7
M	3 54	4 37	23 28	28 69	15 5
S D	0 71	0 74	5 66	5 71	2 4
Controls (n=5)					
M	5 77	6 20	26 36	29 26	21 9
S D	0 89	0 56	1 45	2 13	2 8
p	<0 001	<0 001	n s	n s	<0 001

is an index of renal plasma flow (RPF) and GFR suggests that reduced renal plasma flow is one factor responsible for the low GFR If however the GFR was changed only as a function of renal plasma flow the FF would be constant and the same as in controls Since this was not the case some other factor must also contribute to the low GFR The reduction of the FF will then be a rough index of the effect of this other factor The *ultrafiltration pressure* is determined by the difference between hydrostatic and oncotic pressure forces Decreased hydrostatic pressure i e decreased blood pressure and increased oncotic pressure i e increased serum protein content might thus have resulted in a decreased GFR The arterial blood pressure was reduced but the correlation between FF and the arterial blood pressure was non existent A reduced FF could hardly be due to a reduction of the size of the glomerular capillaries It is therefore suggested that this disorder might by some unknown mechanism alter the *water permeability of the capillary wall* The permeability of the capillary is in part determined by its molecular structure It might therefore be suggested that certain catabolic states where protein deprivation is not predominant (e g AN fasting in obesity) will alter the composition of the changed molecules of the capillary wall so that its water permeability will decrease The good correlation between the severity of the disease and the FF will provide indirect evidence for this hypothesis The edema that sometimes occurs in AN might be explained by reduced facilities for fluid reabsorption from the interstitium to the venous side of the capillaries In that situation edema will occur whenever the endogenous water production will increase Low GFR will of course also contribute to fluid retention

The maximal urine osmolarity was somewhat lower in AN but none of the patients studied had any pronounced reduction of the concentrating capacity As reported previously both in starvation (12) and in AN (14) the administration of vasopressin to fluid deprived patients did not normalize the concentrating capacity This suggests that the defect is of renal rather than of central origin The concentrating capacity of the kidney is generally considered to be dependent on

1 Na^+ reabsorption from the loop of Henle

2 urea recycling from the collecting duct to the medullary interstitium and loop of Henle and

3 osmotic water diffusion from the collecting duct to the renal interstitium and the vasa recta (16)

The nature of the defect is not evident from the present results Low serum urea value is generally accompanied by decreased concentrating capacity (11) but in AN serum urea was actually slightly increased Fractional Na^+ and urea clearance and serum Na^+ did not differ from that found in controls It is therefore possible that reduced water permeability in the collecting duct and/or the vasa recta might also contribute to the defect urinary concentrating capacity in AN

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Table 3 *Sodium and urea excretions in patients with AN*

V = urine flow (volume) C_{In} = glomerular filtration rate n s = non significant

	Sodium serum (mmol/l)	Urinary Na excretion (mmol/hour)	C_{Na}/C_I (%)	Urea nitrogen serum (mmol/l)	C_{urea}/C_{In}[b] (%)
Anorexia					
M	137 3	9 40	1 98	4 82	74 31
S D	2 2	3 62	0 63	1 18	15 76
Controls					
M	137 0	10 49	1 40	3 38	78 17
S D	3 5	2 72	0 47	0 68	17 01
p	n s	n s	n s	<0 01	n s

[a] C_{Na}/C_I fraction of filtered Na that has been excreted is calculated as

$$\frac{\text{urinary Na}\times V}{\text{serum Na}\times C_I}$$

[b] C_{urea}/C_I fraction of filtered urea that has been excreted is calculated as

$$\frac{\text{urinary urea}\times V}{\text{serum urea}\times C_I}$$

reduced GFR and reduced urinary concentrating capacity are generally reported (1 2) In malnourished adults the results are more conflicting (15, 13) which might in part be explained by the heterogeneity of the material studied In contrast with the findings in AN, reduced FF is rarely reported in malnutrition It should however be remembered that the metabolic conditions might differ in AN and malnutrition Protein depletion is probably of greater importance in malnutrition than in AN It is also of interest in this connection that fasting of obese individuals (5) will result in reduction of GFR comparable to that found in AN This reduction in GFR is accompanied by a fall in FF The origin of the reduced GFR in AN malnutrition and fasting obese individuals is still unclear

In the present study an attempt has been made to evaluate which factor or factors that are responsible for the reduced GFR GFR is a function of

1 Renal plasma flow

2 Ultrafiltration pressure in the glomerular capillaries,

3 The filtration coefficient which is the product of the size and the permeability of the glomerular capillaries (4)

The *renal plasma flow* measured as C_{PAH} was reduced in some but not all of the patients Since the cardiac output is also generally reported to be low in AN (7) the decrease of C_{PAH} sometimes recorded is not necessarily due to a specific renal vasoconstriction

The good correlation between C_{PAH} which

Table 4 *Urinary concentrating capacity in patients with AN*

Patients	Max Uosm 17 hr dehydration (mosm/l)	Max Uosm 17 hr dehydration + pitressin (mosm/l)	Increase (%)
CP	820	840	2 4
YN	860	780	−9 3
AN	755	820	8 6
MS	836	880	5 3
ME	420	700	66 7
AS	736	980	33 ?
UP	1 200	1 228	2 3
KÅ	530	550	3 8
GG	705	700	−0 7
M	762	831	
S D	219	193	
Controls (*n*=5)			
M		1 034	
S D		132	

Non significant in comparison to the controls and dehydration without vasopressin

is an index of renal plasma flow (RPF) and GFR suggests that reduced renal plasma flow is one factor responsible for the low GFR If however the GFR was changed only as a function of renal plasma flow the FF would be constant and the same as in controls Since this was not the case some other factor must also contribute to the low GFR The reduction of the FF will then be a rough index of the effect of this other factor The *ultrafiltration pressure* is determined by the difference between hydrostatic and oncotic pressure forces Decreased hydrostatic pressure i e decreased blood pressure and increased oncotic pressure i e increased serum protein content might thus have resulted in a decreased GFR The arterial blood pressure was reduced but the correlation between FF and the arterial blood pressure was non existent A reduced FF could hardly be due to a reduction of the *size of the glomerular capillaries* It is therefore suggested that this disorder might by some unknown mechanism alter the *water permeability of the capillary wall* The permeability of the capillary is in part determined by its molecular structure It might therefore be suggested that certain catabolic states where protein deprivation is not predominant (e g AN fasting in obesity) will alter the composition of the changed molecules of the capillary wall so that its water permeability will decrease The good correlation between the severity of the disease and the FF will provide indirect evidence for this hypothesis The edema that sometimes occurs in AN might be explained by reduced facilities for fluid reabsorption from the interstitium to the venous side of the capillaries In that situation edema will occur whenever the endogenous water production will increase Low GFR will of course also contribute to fluid retention

The maximal urine osmolarity was somewhat lower in AN but none of the patients studied had any pronounced reduction of the concentrating capacity As reported previously both in starvation (12) and in AN (14) the administration of vasopressin to fluid deprived patients did not normalize the concentrating capacity This suggests that the defect is of renal rather than of central origin The concentrating capacity of the kidney is generally considered to be dependent on

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LIMB CIRCULATION IN ANOREXIA NERVOSA

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ABSTRACT Freyschuss U Fohlin L and Thoren C (Departments of Clinical Physiology Karolinska Hospital and Paediatrics St Goran s Hospital Karolinska Institutet Stockholm Sweden) Limb circulation in anorexia nervosa Acta Paediatr Scand 67 225 1978.—Blood flow skin temperature and blood pressure of the lower limbs and the effect of indirect radiant heat on calf blood flow and leg skin temperature was determined in sixteen children with anorexia nervosa (group A) and fourteen healthy children (group H) of the same age and body height Calf blood flow was measured by venous occlusion plethysmography Arm blood pressure was obtained by tourniquet and toe pressure and digital plethysmograms by a strain gauge Skin temperature was measured with a thermocouple In group A calf blood flow was about 50–60 % lower than the mean values observed in group H and a marked difference was maintained after the heat load Skin temperature of the knees and toes were higher in group H Systolic arm blood pressure and toe pressure were on the average 20 mmHg and 13 mmHg lower in group A It is suggested that there is a heat-conserving selective peripheral vasoconstriction in the anorexic patients

KEY WORDS Anorexia nervosa peripheral circulation occlusion plethysmography healthy school children blood pressure temperature regulation digital plethysmography

It is well known that patients with anorexia nervosa (AN) often complain of cold hands and feet (2) The thermoregulation has been reported altered in AN (7 9 12) and in a previous study of the thermal responses to exercise of a group of AN patients (3) it was noted that though core temperature rose normally in a thermal neutral environment during work there was some evidence of peripheral vasoconstriction It was concluded that the patients attempt to limit the size of the body core in order to conserve body heat by maintaining active vasoconstrictor tone in the extremities Under these conditions heat can be exchanged by a counter current mechanism since blood is diverted from the superficial to deep veins which lie in close proximity to the arteries (10) If this theory is correct and anorexia patients adapt to their condition one might expect peripheral blood flow to the limbs to be decreased at rest and show a reduced response to localised heating A selective control of peripheral blood flow was suggested to occur for the compensation of the decreased thermal insulation due to the loss of body fat Wakeling & Russel (12) showed that the oral and finger temperatures of eleven girls with AN responded slowly to localised heat stress imposed by placing the arm up to the elbow in water at 45°C In order to study this problem further we have investigated the effects of localised radiant heat on peripheral (calf) blood flow measured by venous occlusion plethysmography in 16 AN patients

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MATERIAL

The twelve girls and four boys with AN (group A) who were investigated belonged to a larger series which has

Table 1 *Physical characteristics of sixteen anorexic (group A) and fourteen healthy children (group H)*

Mean values ±S D and significance of inter group differences are given

	A	Sign	H
Age years	15 2±2 0	NS	14 7±1 1
Height cm	164±9 8	NS	164±9 4
Weight kg	37 5±7 3	$p<0\ 001$	49 1±8 5

been presented elsewhere (1 6) Healthy controls (group H) ten girls and four boys were selected by age height and sex to match the anorexic children Physical characteristics of the groups are given in Table 1 Among the anorexic children the girls had on an average less than 10% of their body weight as fat compared with less than 5% in the boys The mean percentage weight loss was 27 in the girls and 24 in the boys and the disease had lasted for about one year in all the patients As observed in the larger series (6) they had a resting bradycardia about 52 beats per min

METHODS AND PROCEDURE

Calf blood flow was measured by venous occlusion plethysmography with an air filled plethysmograph according to Dohn (4) and Graf & Westersten (8) the plethysmograph cuff being placed around the thickest part of the calf Venous occlusion pressure was 50 mmHg During the recording of inflow curves the circulation of the foot was occluded by an ankle cuff inflated to 240 mmHg The inflow curves were recorded on a Mingograph 81 (Siemens Elema) Blood flow was calculated in ml×min $^{-1}$ ×100 ml tissue $^{-1}$

Digital pulse plethysmography The arterial volume pulsations fo the first toe were recorded by a strain gauge The pulse curves were recorded with a Mingograph (Siemens Elema Sweden) and submitted to a shape analysis with regard to propagation and inclination times ECG was recorded simultaneously

Blood pressure was measured with a tourniquet around the arm toe pressure was obtained by strain gauge

Skin temperature at 5–6 levels from the knees to the toes was recorded before and after indirect heating by means of a thermocouple connected to a direct reeding mirror galvanometer (TE 3 Ellab Denmark) Temperature was registrated with an accuracy of ±0 1 C The temperature of the subjects right and left leg were similar

All measurements were first performed at room temperature and then repeated following 30 min of radiant heating The heating lamps were encased in a box which surrounded the abdomen All recordings were made with the patients in supine position

Current statistical methods were applied to the data and comparisons were made on an intergroup basis The probability (p) levels of significance are indicated as $p<0\ 001$ highly significant (***) $p<0\ 01$ significant (**) and $p<0\ 05$ probably significant (*)

RESULTS

The mean values of the circulatory data in the anorexic and healthy children and the difference between the two groups are given in Tables 2 and 3

In group H data of resting calf blood flow and inclination time in the digital plethysmogram were similar to the composite material of healthy adults and children described by Thulesius & Wahren (11)

In group A calf blood flow was slightly less than half ($p<0\ 001$) of the mean values observed in group H (Table 2) and a marked difference ($p<0\ 001$) was maintained after exposure to indirect radiant heat In the latter situation limb blood flow increased significantly in both groups but more so among the healthy children the absolute flow increase being about three times greater than in group A The digital pulse plethysmogram and its subdivisions i e propagation and inclination time were similar to the anorexic and healthy children Systolic arm blood pressure was on the average 20 mmHg lower ($p<0\ 001$) in group A while there was no difference in diastolic pressure A lower toe blood pressure (by about 13 mmHg $p<0\ 05$) was also found in

Table 2 *Circulatory data of 16 anorexic (A) and 14 healthy children (H)*

Mean values ±S D and significance of inter group differences are given

	A	Sign	H
Calf blood flow ml min $^{-1}$ 100 ml $^{-1}$			
Before			
Right	2 0±1 0	***	4 3±1 6
Left	1 9±0 8	*	4 4±1 8
After indirect heat			
Right	3 0±1 7	**	7 5±3 5
Left	3 1±1 3		7 3±3 0
Blood pressure mmHg			
Arm	94/65	** /NS	114/69
Toe right	77±14		90±18
Toe left	79±19	*	93±17
Digital pulse plethysmogram			
Propagation time csek	34	NS	33
Inclination time csek	11	NS	11

Table 3 *Skin temperature before and after in direct heating in anorexic (A) and healthy children (H)*

Mean values ±S D and significance of inter group dif ferences are presented

	A	Sign	H
Temperature C°			
Knee	30 0±1 6		32 1±1 2
Toe	27 1±4 0		32 4±1 4
Diff knee–toe	2 6±3 6		−0 2±0 9
Temp after heating C°			
Knee	35 1±2 2		36 4±0 6
Toe	32 1±4 3		35 2±1 9
Diff knee–toe C°	2 4±2 9	NS	1 3±1 9

group A In accordance with the limb flows skin temperature over the knees and toes were higher in the healthy children both before and after heat exposure (Table 3)

DISCUSSION

A decreased peripheral blood flow and a low ered skin temperature have been demon strated in this investigation This accords with the common clinical finding of cold hands and feet in patients suffering from anorexia ner vosa The disorder seems to effect both the calf blood flow at normal laboratory tempera ture and the peripheral circulatory response to indirect heat exposure The decline in blood flow cannot be solely explained by muscular wastage and associated changes in limb seg ment composition in the A group as a signifi cant difference is maintained if the plethysmo-graphic measurements are corrected for the loss of muscular volume according to Thorén & Wahren (11)

In previous studies of the larger series of AN a low oxygen uptake at rest (6) and a low rectal temperature (3) were found In all of them in whom cardiac output was determined the central circulation was normokinetic (5 6) i e cardiac output was related to the oxy gen uptake as in healthy children The low limb flow values might then be proportionate to the cardiac output provided the latter is nor mally distributed to different vascular beds However a need to restrict the amount of heat lost from the body surface has characterized anorexic children previously investigated (3) Thus the skin temperatures (T_{sk}) of the fingers and hands of some patients were maintained at a lower level than the T_{sk} values of their head and trunk and at 13 C below the T_{sk} of the extremities of control subjects exercising un der the same environmental conditions The thermal responses to prolonged exercise (3) demonstrate that the anorexic children ther moregulate adequately in thermally neutral en vironment but less adequate in cold or warm conditions due to the loss of thermal insula tion

The impaired circulatory response to in direct heat exposure in the present study indi cates an increased peripheral vascular tone Therefore the decline in limb blood flow is probably due to an altered distribution of car diac output in turn due to a peripheral vascu lar constriction A lower oral and arm skin temperature before and after immersion of the contralateral forearm in warm water was re corded in anorexic females by Wakeling & Russel (12) They admitted that an increased peripheral vascular tone might be a conceiv able way to conserve core temperature but found the mechanism exaggerated due to its persistence after heat load and in rehabilitated patients The latter argument can be criticized as the recovered patients had not regained either their initial or predicted standard weights at the time of the investigation and the temperature reaction on caloric load was more quantitatively than qualitatively changed

The pulse plethysmograms were similar in both groups of children This is to be expected when the basis of the circulatory malfunction is functional rather than organic as in periph eral arterial diseases It might be concluded that the circulatory pattern is well adapted to conserve heat in patients with a decreased thermal insulation

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TOTAL BODY POTASSIUM FAT FREE WEIGHT AND MAXIMAL AEROBIC POWER IN CHILDREN WITH ANOREXIA NERVOSA

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ABSTRACT Davies C T M von Dobeln W Fohlin L Freyschuss U and Thoren C (The Department of Paediatrics Karolinska Institutet at ST Goran s Children s Hospital and the Aeromedical Laboratory of the Swedish Medical Research Council at Gymnastik och Idrottshögskolan Stockholm Sweden) Total body potassium fat free weight and maximal aerobic power in children with anorexia nervosa Acta Paediatr Scand 67 229 1978.—Body composition and aerobic work performance have been studied in 5 boys and [illegible] girls suffering from anorexia nervosa The average ages of the two groups of children were 15 4 (boys) and 15 2 (girls) years respectively Measurements of body composition included height weight (W) body potassium (^{40}K) skinfold thickness (SFT) at triceps and subscapularis blood volume (BV) and femoral condylar and radioulnar breadths From these measurements estimates of fat free weight (FFW) skeletal weight (S) and lean body mass (LBM) were made Work performance was assessed by measurement of the maximal aerobic power ($\dot{V}_{O_2}$ max)

The patients had lost on average 26% of their former body weight The boys had on average >7% of their body weight as fat compared with >9% [illegible] the girls However the loss of weight was not solely due to loss of body fat but could also be ascribed to a decrease in soft fatfree tissue LBM or FFW could be estimated as well from SFT as from ^{40}K $\dot{V}_{O_2}$ max averaged 1 43 l/min (35 1 ml/kg/min) in the anorexic boys and 1 24 l/min (33 2 ml/kg/min) in the girls and was associated with FFW and LBM However $\dot{V}_{O_2}$ max was lower in relation to LBM than in healthy children of the same age Thus it was suggested that the emaciation in anorexia is directly attributable [illegible] loss of both fat and muscle and accounts in part for the reduction of aerobic power observed However an important factor may be the debilitating effect of starvation on the patient particularly in its advanced and later stages which reduces his/her level of habitual physical activity

KEY WORDS Anorexia nervosa body composition children exercise lean body mass maximal oxygen uptake

There have been several studies of body composition in children (13 28 31) young healthy adults (12 19 34 35) and obese patients (6 15) and the development of the total body potassium (^{40}K) method for estimating fat free weight (FFW) has increased our knowledge of body structure in health (4 5) and disease (3 23 29) The effect of severe caloric deprivation has been documented in civilians and prisoners of war (26) under carefully controlled laboratory conditions in young healthy adults (26) and [illegible] patients undergoing restricted diet treatment for obesity (25) and in adult patients with anorexia nervosa (20 27)

Nevertheless there still remains [illegible] lack of information of diet starvation on body structure and physiological function in the pubescent child In the present investigation

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Table 1 *Body composition Blood volume and maximal oxygen uptake in anorexia nervosa patients*

Mean ±S [illegible] Abbreviations see text Within brackets the number of patients when less than the series

	Age (y)	Ht (cm)	W (kg)	Weight loss (%)	LBM (kg)	K (g)	S (kg)
Boys=5	15 4±2 5	169±17	40 8±10 1	23±4	38 0±8 5	81 5±11 8	10 3±
Girls=10	15 2±1 9	163±8	36 1± 6 4	28±7	32 8±4 7	75 7±11 4	8 ?±

we have examined body composition includ ing measurements of ^{40}K in 15 patients suffer ing from anorexia nervosa and related our find ings to maximal aerobic power (V_{O_2} max) measured on a bicycle ergometer

MATERIAL AND METHODS

The material for this investigation was drawn from a series of anorexia nervosa (AN) patients on whom measure ments of thermoregulatory function and the dimensions of the O_2 transporting system have been previously reported (14 21) In the present study 15 patients (10 girls and 5 boys) were measured on separate occasions All patients selected for this study conformed to the following criteria formulated previously (21) for AN

1 Age at onset less than 25 years
2 Active refusal to eat with accompanying pronounced weight loss
3 No evidence of schizophrenia severe depression or organic disease

Amenorrhoea was present in all the post pubescent female patients and none of the patients had edema The *weight height* and *skinfold thickness* were measured using a clinical balance accurate to ±100 [illegible] a stadiometer accu rate to ±1 cm and Harpenden skinfold caliper accurate to ±5% respectively

From the sum of the two skinfolds triceps and sub scapularis *lean body mass (LBM)* was calculated accord ing to Pařízková (30) A multi stage *exercise test* was performed on an electrical braked bicycle ergometer (Siemens ELEMA) with stepwise increased work loads up to maximal level i e to the limit of their exercise capacity *Oxygen uptake* was determined from expired air collected successively in Douglas bags during the last minutes of the final work loads Expired air volume was determined by evacuating the bag through a Tissot spirometer and O_2 and CO_2 contents were measured by the micro Scholander technique *Blood lactate* (LA) was determined immediately and 4 min after cessation of exer cise from pre warmed (arterlialised) finger prick sam ple after an enzymatic method (10) The criteria for maxi mal *aerobic power* ($\dot{V}_{O_2}$ max) having been reached were that LA should exceed 9 mM/l and respiratory quotient should exceed unity (2)

Whole body potassium (^{40}K) was determined in a whole body counter on the aeromedical laboratory The countr of the gamma emission from the body of the subject w performed within a chamber with lead walls [illegible] mm thic The subject was sitting reclined in a standard chair with a angle of 90° between the body and the thigh In the cent line of this angle the center of a plastic scintillator of t size 20×10×6 5 inches was placed The signals from t photomultipliers attached to the plastic scintillator we analyzed in a 100-channel analyzer Counting time w 2400 s

The calibration of the gamma counter had earlier be done using male and female adult subjects whose bo composition was known from density determination l hydrostatic weighing Their potassium content was es mated using the values given by Forbes & Lewis (22) T total error of the method was found to be between 5 a 6% The statistical error derived from Poisson s law for subjects with 130 g potassium 2 2%

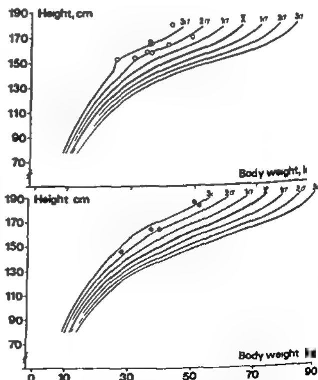


Fig 1 Relationship of weight to height in anorexic girls and boys The standard height–weight curves of normal healthy Swedish children are shown Girls (O) boys (●)

?W (kg)	BV (l)	$\dot{V}O_2$ (l/min)
1±6	3 16±0 64	1 43±0 3?
1 9±3 9	? 76±0 49	1 ?4±0 19 (8)

Using the potassium content of the body the weight of the soft fatfree tissue of the test subject was estimated from the formula

$$ST = \frac{K - 1\,1S}{2\,7}$$

where ST = soft fatfree tissue (kg) K = g potassium from gamma emission S = weight of skeleton (kg) from measurements of body height the sum of right and left condylar breadths and the sum of the right and left radioulnar breadths using the empirical formula developed by von Döbeln (17 18) The constants represent the normal potassium concentration of the skeleton and the soft fatfree tissue respectively Fatfree weight (FFW) was derived from the sum of ST and S (17 18)

RESULTS

The basic data are summarized in Table 1 The patients are approximately −3 S D below their expected weight for height (Fig 1) Calculated from skinfold thickness the boys had 6 4 ±2 5% of their total body weight as fat the corresponding figure in the girls is 8 5 ±3 5% Skinfold thickness at triceps and subscapularis average 3 4 and 4 0 mm for the boys and 5 4 and 4 5 mm for the girls These values are well outside the lower normal range given by Tanner (32) (Fig 2) In absolute values ^{40}K was reduced compared to normal children of the same age but in terms of body weight ^{40}K was 2 12 g/kg in the boys and 2 10 g/kg in the girls There were significant correlations between ^{40}K and body weight with a coefficient (r) of 0 97 and 0 80 for the boys and girls respectively The regression equation relating ^{40}K to body weight height and LBM for the male and female patient are given in Table 2 A highly significant relationship of FFW estimated from ^{40}K and skeletal weight to LBM determined from skinfold thickness was found (Fig 3) However by the method of calculation of FFW it was not possible to detect any fat at all in two patients in whom the skinfold thickness gave a result of about 4% fat Skeletal weight accounted for on average 25% of the body weight in the boys and 23% in the girls The corresponding mean values for healthy young male and female subjects are 19% and 17% (16) There was also a significant (r=0 97) relationship between V_{O_2} max and LBM (Fig 4)

DISCUSSION

Interest in the field of human starvation was stimulated by the aftermath of World War II It

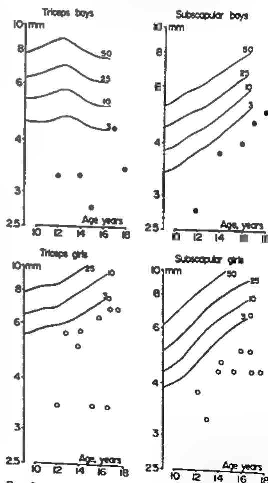


Fig 2 Triceps and subscapular skinfolds in anorexia nervosa patients 50th 25th 10th and 3rd percentile from Tanner et al (3)

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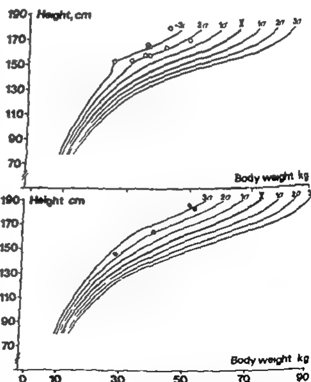


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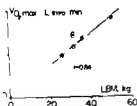


Fig 4 Maximal oxygen uptake ($\dot{V}o_2$ max) in relation to LBM estimated from skinfold thickness Symbols as Fig 1 $\dot{V}o_2$ max=0 035+0 036 LBM [illegible]<0 001

age values and are open to some criticisms as are our own estimates which were made on a young but homogenous population of normal men and women and includes estimates of skeletal weight The use of either their or our constants for the FFW equivalent of ^{40}K does not materially alter the two sets of data and the difference between the absolute values of ^{40}K in Edmonds et al s anorexic female patients and our own remain obscure The calculation of LBM could also be critisized since it was derived from a normal material of 8 to 12 year-old children However there were no statistically significant difference between LBM calculated from the sum of two or ten skinfold thicknesses derived from children of similar age (30) The prediction of LBM from skinfold measurements in this study is in good agreement with other studies in young children (?8) and in adults (5)

The loss of body weight and LBM is accompanied by a reduction in physiological work performance in AN In the male and female patients absolute Vo_2 max is decreased by approximately 45 and 40% respectively when compared to normal children of the same age (33) If account is taken of body weight the difference between normals and patients is reduced to 30% for the boys and 20% for the girls In relation to LBM the reduction however still seems to be of the order of 40% in the boys and 30% in the girls (30) with a mean value of 37 6 ml and 37 8 ml/kg LBM in boys and girls respectively The decrease of Vo_2 max [illegible] therefore greater than can be attributed to their change in body size and composition However some caution is necessary when interpreting data for Vo_2 max In the present study it was not always possible to apply the accepted criterion for Vo_2 max namely a plateau of Vo_2 on work load at exhaustion However the patients were encouraged to pedal the ergometer at maximal effort and the high levels of lactic acid concentration in their blood (21) bear witness to the fact that they did exercise to exhaustion and were at or very close to their true Vo_2 max The decrease in aerobic power [illegible] also in good agreement with results obtained from experimantal starvation (26) Undoubtedly as the disease progresses and particularly during its later stages AN has a debilitating effect on the patients which must contribute to their low aerobic capacity This in turn reduces their exercise tolerance still further and possibly give rise to a situation as found in the present investigation where Vo_2 max decreases out of proportion to changes in body composition and as shown by Fohlin et al (21) to the dimensions of the O_2 transporting system

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Table 2 *Regression equations for whole body potassium (^{40}K) on body weight height and lean body mass (LBM) calculated from skinfold thickness in anorexia nervosa patients*

b is the regression coefficients of *x* *a* is the constant term *r* the correlation coefficient and *n* the number of patients

	n	*y*	*b*	*x*	*a*	*r*	*p*
Boys	5	K	1 23	Weight	33 3	0 97	<0 01
Girls	10	K	1 41	Weight	24 6	0 80	<0 01
Boys	5	K	0 76	Height	−45 3	0 98	<0 01
Girls	10	K	0 63	Height	−27 1	0 45	>0 05
Boys	5	K	1 48	LBM	27 2	0 97	<0 01
Girls	10	K	1 84	LBM	15 0	0 75	<0 05

culminated in the classic Minnesota experiment from 1950 (26) which still remains today the standard reference for the biology of human starvation though the study was confined to young adult men AN is a severe form of starvation which differs in several respects from involuntary starvation (11) AN patients do eat small amount of food selectively there are no signs of protein malnutrition haemoglobin levels are usually normal and symptoms of vitamin deficiency diseases are rare The degree of starvation or weight loss in our series are comparable or greater than those found in either famine victims or the laboratory experiments on semistarvation (26) The decrease of body weight in AN is not solely due to the loss of fat as our measurements of ^{40}K and estimates of FFW and LBM indicate (Table 1) In absolute terms ^{40}K LBM and BV are markedly reduced in the patients compared with normal children of the same age but it should be noted that the reduction of BV and ^{40}K is in relation to their loss of body weight The mean figure of 78 ml BV per kg of body weight compares favourably with normal data for healthy children (24) A similar proportion of diminuition of exchangeable potassium has been reported in adult patients (27) and in malnourished children (29) K is linearly associated with weight (Table 2) Similar relationship can be obtained from our data for BV ($r=0\ 60$ $p<0\ 05$) Further it would seem (Fig 3) that the degree of emaciation in terms of loss of LBM or FFW can be estimated both from simple skinfold measurments and from determinations of ^{40}K This is contrary to the view recently reported by Edmonds et al (20) They found in 12 malnourished adult patients 7 of whom had established AN that FFW determined from ^{40}K was low and the calculated fat values were high (range 20–50% body weight) and bordering on those found for the obese It is difficult to reconcile their observations with our own results It is well known that age sex and physical fitness (8 9) have an effect on ^{40}K and it is to be expected that ^{40}K will be reduced in AN as we and Edmonds et al have found due to loss of cellular mass Our ^{40}K values expressed in g per kg body weight are 2 12 for the boys and 2 10 for the girls and compare favourably well with those of others (1 7 19) The validity of the determination of FFW from ^{40}K of course depends on the calculations used to obtain potassium content of human lean tissue Edmonds et al used aver

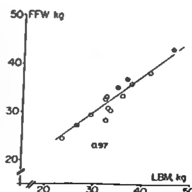


Fig 3 Fat free weight (FFW) estimated from measurements of ^{40}K in relation to lean body mass (LBM) estimated from skinfold thickness Symbols as Fig 1 FFW=6 08+0 80 LBM $p<0\ 001$

Acta Pædiatr Scand 67 235–236 1978

LETTER TO THE EDITOR

Sir

I write to disagree with Dr Bjarke whose letter concerning palpation of the femoral pulse was published in the March issue of Acta Paediatr Scand 1977 I believe he has fallen into the same trap as the text books he quotes Like them he stresses the importance of pal pation of periferal (sic) pulses and then proceeds to talk only of the femoral Assuming the object of the exercise is to suspect the diagnosis coarctation of the aorta (which is then confirmed with blood pressure record ings) I believe that paediatricians would be better advised to forget the femoral and really palpate the peripheral In this context this means the tibial pulses at the ankle—re membering of course that not all legs have an anterior tibial artery (1) I have several reasons for this

(1) The tibial arteries are subcutaneous at the ankle (1) and provided that the examiner's hand and patient s foot are warm are *easily* palpable in all normal neonates and children The same is not true of the femoral artery—hence Dr Bjarke s letter

(2) In my experience no child with coarcta tion under four years old has palpable tibial pulses i e the observation is all or nothing This is not true of the femoral pulse—even very young patients with coarctation may have palpable femoral pulses The text book advice is then that the examiner should detect delay between the brachial and femoral pulses This is a counsel of perfection when applied to the very young Detection of delay may be possible when the pulse rate is 80—I do not believe it is possible when the pulse rate is 150

(3) To adequately feel the femoral pulse it is often necessary to abduct and externally rotate the leg—the young don t like it and cry!

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The Editor has asked Dr Bjarke to comment on Dr Burnell s letter

Sir

I welcome Dr Burnell s interesting letter We seem to share the same opinion about the im portance of palpating the arterial pulse in the newborn and the difficulties sometimes en countered by the physician

Dr Burnell describes a method which he claims is superior to the traditional palpation of the femoral artery in detection of a coarcta tation of the aorta He suggests that pae diatricians would be better advised to forget the femoral and really palpate the peripheral

I think this is an unlucky suggestion The aim of the palpation of the femoral artery is not just to try to detect the presence of a coarcta tion of the aorta but also to get an indication of the situation in the *central* circulation More information than is often recognized can

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SHORT COMMUNICATION

C3 IN HUMAN MILK

Breast feeding is known to protect the infant against infection Several anti bacterial factors have been detected in human milk Our earlier studies in Indian women have shown that breast milk contains significant amounts of secretory IgA lysozyme and lactoferrin and that the concentration of these factors is not influenced by the nutritional status of the mother (5) Recently we have extended these studies to obtain information on the concentration of C3 component of the complement system in human milk

Samples of breast milk were collected from 168 women at various stages of lactation They were classified into two groups—well nourished and undernourished based on their weight and socio-economic status C3 concentration in milk was determined by the radial immunodiffusion method (3) The results are presented in Table 1 The concentration of C3 was found to be high in colostrum and showed a rapid fall during the first month of lactation reaching a mean level of 0 16 g/l in mature milk There was not much change in the concentration during the subsequent periods of lactation There were no significant differences in the levels of C3 in milk samples obtained from well nourished and undernourished women

Complement is a complex system comprising a series of proteins of which C3 is an important component It plays an important role in bacteriolysis Although various components of serum complement have been investigated extensively (2) there are few studies on these components in milk (4) It has been suggested that C3 in milk can be activated through the alternate pathway in addition to the classical route due to the high concentration of secretory IgA in milk (1) Data presented here show that the concentration of C3 in milk is about 20% of that present in serum and nutritional status of the mother does not influence the concentration These observations are in line with our earlier studies (5) and indicate that breast milk contains significant amounts of C3 in addition to other antibacterial factors These qualities of breast milk are of major importance for the infant s defence against infection particularly in developing countries where the risk of infection is high

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Table 1 *C3 concentration in human milk*
Values are mean ± S E

Sample	Undernourished women No	Undernourished women C3 level (g/l)	Well nourished women No	Well nourished women C3 level (g/l)
Colostrum (1–5 days)	13	0 25±0 009	15	0 33±0 02
Transition milk (6–30 days)	2[illegible]	0 [illegible]0±0 00[illegible]	37	0 22±0 01
Mature milk (>30 days)	61	0 17±0 001	25	0 16±0 00[illegible]

be gained from the quality of the pulse. Critical aortic stenosis, hypoplastic left heart syndrome, total anomalous venous drainage etc. with low cardiac output might be indicated by a weak pulse. Bounding pulses are found in patients with truncus arteriosus and children with ductus arteriosus with large left to right shunts.

What we actually palpate is the quality of the pulse wave at the point of palpation, knowing that the original pulse wave becomes progressively distorted as it travels towards the periphery. Since the object is to evaluate changes imposed on the pulse wave either by functional or anatomical alternations in the central circulation it seems logic to palpate the arterial pulse as centrally as possible rather than in the periphery.

In my letter I described a technique found *to have facilitated the palpation of the femoral* artery. There is no need to abduct or rotate the leg externally and the pulse is quite readily located. Hence points 1 and 3 in Dr Burnell's letter seem less relevant.

I agree that it is asking too much of th[e] physician to expect him to recognize a pulse delay between the brachial and femoral artery. The advice should be to compare the magni[-]tude of the pulses, not the time relationship.

The inconsistency of the femoral pulses i[n] the neonate with coarctation of the aorta is recognized by paediatric cardiologists (1, 2). In fact the femoral pulse may be quite normal. I see no reason why the pulse wave in such cases should not be transmitted to the tibial arteries and there thus being palpable. It is therefore hard to believe that palpation of the tibila arteries is the all or nothing observation claimed.

Bjorn Bjarke

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SHORT COMMUNICATION

C3 IN HUMAN MILK

Breast feeding is known to protect the infant against infection Several anti bacterial factors have been detected in human milk Our earlier studies in Indian women have shown that breast milk contains significant amounts of secretory IgA lysozyme and lactoferrin and that the concentration of these factors is not influenced by the nutritional status of the mother (5) Recently we have extended these studies to obtain information on the concentration of C3 component of the complement system in human milk

Samples of breast milk were collected from 168 women at various stages of lactation They were classified into two groups—well nourished and undernourished based on their weight and socio economic status C3 concentration in milk was determined by the radial immunodiffusion method (3) The results are presented in Table 1 The concentration of C3 was found to be high in colostrum and showed a rapid fall during the first month of lactation reaching a mean level of 0 16 g/l in mature milk There was not much change in the concentration during the subsequent periods of lactation There were no significant differences in the levels of C3 in milk samples obtained from well nourished and undernourished women

Complement is a complex system comprising a series of proteins of which C3 is an important component It plays an important role in bacteriolysis Although various components of serum complement have been investigated extensively (2) there are few studies on these components in milk (4) It has been suggested that C3 in milk can be activated through the alternate pathway in addition to the classical route due to the high concentration of secretory IgA in milk (1) Data presented here show that the concentration of C3 in milk is about 20% of that present in serum and nutritional status of the mother does not influence the concentration These observations are in line with our earlier studies (5) and indicate that breast milk contains significant amounts of C3 in addition to other antibacterial factors These qualities of breast milk are of major importance for the infant s defence against infection particularly in developing countries where the risk of infection is high

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Table 1 *C3 concentration in human milk*
Values are mean ± S E

Sample	Undernourished women No	C3 level (g/l)	Well nourished women No	C3 level (g/l)
Colostrum (1–5 days)	13	0 25±0 009	[illegible]	0 33±0 0[illegible]
Transition milk (6–30 days)	[illegible]	0 [illegible]0±0 002	32	0 22±0 01
Mature milk (>30 days)	61	0 17±0 001	25	0 16±0 001

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CASE REPORT

HYDROPS FETALIS HYDRAMNIOS AND HEPATIC VASCULAR MALFORMATION ASSOCIATED WITH CUTANEOUS HEMANGIOMA AND CHORIOANGIOMA

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From the Department of Pediatrics and the Department of Pathology Bellevue New York University Medical Center New York N Y USA

ABSTRACT Shturman Ellstein R Greco M A Myrie C and Goldman E K (Dept of Pediatrics and Department of Pathology Bellevue New York University Medical Center New York N Y) Hydrops fetalis hydramnios and hepatic vascular malformation associated with cutaneous hemangioma and chorioangioma Acta Paediatr Scand 67 239 1978.—A premature baby presented with severe hydrops fetalis due to a multifocal angiomatous malformation of the liver There were two other small vascular tumors hemangioma of the skin and chorioangioma Hydramnios and placental edema were also present The association of severe hydrops fetalis and hydramnios with angiomatous malformation of the liver was not found in reviewing the literature

KEY WORDS Hydrops fetalis hydramnios hepatic vascular malformation

Hydrops fetalis edema of the placenta and hydramnios have been reported in association with many maternal and fetal conditions (9 12 15 16) Among these are included fetal vascular malformations such as chorioangiomas (10 18 20) and on one occasion there was an abdominal hemangioendothelioma of the fetus in which no maternal hydramnios was recognized (2) The association of hydrops hydramnios and edema of the placenta with vascular malformations of the liver has not been previously reported

We describe here a case of hydrops fetalis edema of the placenta and hydramnios due to a multifocal angiomatous malformation of the liver associated with a small hemangioma of the skin and a small chorioangioma

CASE REPORT

A 4000 g white female was born after 35 weeks gestation to a 31 year-old apparently healthy woman gravida 4 para 1 aborta 2 blood type A Rh positive The pregnancy was uncomplicated until the 33rd week when marked enlargement of the uterus and an eight pound weight gain was noted X rays of the abdomen confirmed the presence of a single fetus in vertex position as well as evidence of hydramnios

Labor started spontaneously at 35 weeks gestation with partial rupture of the membranes 5 hours prior to delivery The amniotic fluid was clear and copious (approximately 3500 ml) Vaginal delivery of a severely hydropic female infant occurred Apgar scores at 1 and 5 minutes were 1 and 4 The infant required endotracheal intubation administration of oxygen by positive pressure and sodium bicarbonate intravenously

The physical examination revealed a depressed severely edematous and cyanotic newborn in acute distress requiring respiratory assistance The heart rate was 180/minute and temperature 97°F Her weight was 4000 g length 47 cm head circumference 35 cm chest circumference 40 cm The estimated gestational age by physical examination was 36 weeks

There was poor air exchange and scattered rales over both lung fields Auscultation of the heart revealed a gallop rhythm a grade 3/6 systolic murmur The abdomen was very distended and tense There was a 1 2×0 9×0 4 cm cutaneous hemangioma over the right upper quadrant The liver was palpable 4 to 5 cm below the right costal margin No bruit was heard over the liver

X rays revealed severe cardiomegaly hazy lung fields



Fig 1 The liver shows depressed fibrotic areas and engorged blood vessels The heart is dilated and focal hemorrhages are present in the epicardium

blunting of the costophrenic angles free fluid in the abdomen and markedly thickened soft tissues throughout the body The electrocardiogram showed predominant left forces The blood count revealed a hemoglobin of 16 5 g/100 ml hematocrit 51% reticulocytes 4 6% 17 500 white blood cells and 37 000 platelets per µl Erythrocyte morphology was normal Blood type A Rh positive and direct Coombs test negative The mother s platelet count was normal The total protein was 3 3 g/100 ml and albumin 1 8 g/100 ml The central venous pressure was 28 cm of H_2O

Phlebotomy and restoration of the red blood cells was immediately done The patient was treated with digoxin furosemide salt poor albumin and she was placed on a Bourns respirator Shortly thereafter she developed a left pneumothorax which resolved after a chest tube was inserted She developed hematuria a generalized petechial rash and bleeding from puncture sites endotracheal tube and umbilical stump was noted Heparin therapy was given because of laboratory confirmation of disseminated intravascular coagulopathy The platelet count fel 14 000 per cubic mm and the hematocrit dropped to despite continued transfusions The urinary out,ut mained poor During the second day of life despite tilatory assistance she developed severe hypoxa respiratory acidosis and expired

Post mortem examination

The body of this 2-day-old premature white weighed 3 150 g falsely increased due to the anasar cutaneous hemangioma measuring 1 ?×0 9×0 4 cm present in the right upper abdomen The skin and se surfaces displayed multiple petechiae Ascites (9 and hydrothoraces (5–10 ml each) were present The appeared enlarged The hepatic surface was diff hemorrhagic Multiple purplish red irregularly shape pressed areas were scattered over the surface an gorged blood vessels were seen (Fig 1) On cut su those depressed areas corresponded to irregularly sh scars which were not encapsulated (Fig 2) Tiny b filled cystic spaces were noted in some of them umbilical hepatic and portal veins the ductus ve and hepatic artery were normal

Post mortem X ray of the liver showed focal cal tion in two of the fibrotic areas In all fibrotic are plethora of pleomorphic single layered endothelial channels of variable size were embedded (Fig 3 elastic lamina was absent from most of the cha Arteries with irregular muscular coats were seen in areas but serial sections failed to demonstrate ar venous communications The vessels were separat fibrous tissue septae in which remnants of hepatc were present and there was a proliferation of bile Only occasional channels showed proliferatic endothelial cells thromboses and occlusion of some vascular lumina by granulation tissue The prese extensive fibrosis suggested focal spontaneous regr as seen in vascular tumors in children (4 5) No c atypia was noted in any area of the malform Erythrophagocytosis was identified in a few Kupffe in the dilated sinusoids of the liver but not in the



Fig 2 The cut surface of the liver shows the depr areas in continuity with fibrotic areas in the parench Purplish areas of discoloration simulating hemorrhag necrosis were found to contain irregularly dilated cular channels



Fig 3 Histologic section of liver showing large vascular channels and a focal area of proliferation of endothelial cells (left side of the picture) (H & E ×350)

thelial cells Many foci of extramedullary hematopoiesis were present scattered throughout the liver and spleen The heart was dilated and the sections from the left ventricle demonstrated myocardial hypertrophy No structural anomalies were present in the heart or great vessels Both lungs were severely congested and hemorrhagic and hyaline membrane disease was present Other organs were unremarkable except for congestion and focal hemorrhages There was marked vacuolization of the fetal adrenal cortex This has been observed in association with hydropic hemolytic disease but its significance is unknown (6) The bone marrow showed erythroid hyperplasia

The placenta was large and edematous with hypertrophic and hyperplastic villi Its weight was 1090 g A 1 0×0 8 cm well-demarcated tumor was present Small blood filled spaces were identified on cut surface Microscopically the tumor was formed by vascular channels embedded in a myxomatous matrix The external surface of the tumor was lined by trophoblast

In summary the main pathological findings were multifocal angiomatous malformation of the liver capillary hemangioma of the skin chorioangioma hydrops fetalis mild hypertrophy of the left heart and hyaline membrane disease

DISCUSSION

The coexistence of cutaneous and hepatic hemangiomas has been reported many times (4 13 19) and the association of vascular tumors in both the placenta and the fetus has occasionally been reported (17 3) The simultaneous occurrence of a large chorioangioma and hepatic hemangioma was reported in one case (1) and suspected although not confirmed in two cases (17) but none of them with the clinical presentation we have described

In our case a multifocal angiomatous malformation of the liver a single small cutaneous hemangioma and a small chorioangioma were associated with hydrops fetalis edema of the placenta hydramnios and premature delivery The skin and placental lesions were too small to have been responsible for the complications

and there was such extensive involvement of the liver to suggest that the hepatic vascular tumor was the only anatomic basis for the clinical picture

Benign vascular tumors are among the most common tumors of infancy They may affect any organ most commonly the skin and they may be single or multiple In some cases, two or more different tissues contain multiple hemangiomas and the presence of similar lesions in different organs is explained as being multicentric in origin and not as metastases (21) When they are present in the placenta and the fetus they are interpreted as being an abnormal development of the fetal vasculature common to both (17)

Congestive heart failure and hematologic abnormalities have been reported among the serious complications of large vascular tumors including chorangiomas (7) Vascular tumors may function as arteriovenous shunts leading to high output cardiac failure which in fetal life may present as hydrops (2) The shunting affects the placental low pressure system and may cause edema of the placenta and hydramnios and hypoalbuminemia may be a complication of hydramnios (8)

Thrombocytopenia may be observed in vascular tumors (Kasabach Merritt syndrome) Platelet sequestration may result in disseminated intravascular coagulation by stimulation of the coagulation cascade enhanced by the phospholipid surface of the platelets (11) Vascular endothelium contains thromboplastic substances and thrombus formation in the abnormal capillaries may be the result of the release of such thromboplastic substances (10) Although metabolic and respiratory acidosis due to severe heart failure and hyaline membrane disease could be implicated as the cause of disseminated intravascular coagulation the extent of the hepatic hemangioma in this patient probably contributed significantly

The presenting clinical picture of this neonate in combination with a cutaneous hemangioma made us suspect hemangiomatous involvement of other organs Confirmation by angiography was entertained but could not be done

Vascular tumors may represent a medical emergency in infants and children surgery and steroids have been reported as modes of therapy (13 19 14) However, their potential value in this case is questionable

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CASE REPORT

HAEMORRHAGIC INFARCTION OF THE MYOCARDIUM IN A NEWBORN WITH HAEMOGLOBIN H DISEASE AND ERYTHROBLASTOSIS

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From the Department of Paediatrics and the Cardiac Laboratory The Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel

ABSTRACT Gadoth N Kornmehl P Gueron M and Moses S W (Dept of Paediatrics and the Cardiac Laboratory The Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel) Haemorrhagic infarction of the myocardium in a newborn with haemoglobin H disease and erythroblastosis Acta Paediatr Scand 67 245 1978 —Extensive haemorrhagic myocardial infarction developed in a new born apparently as a result of anoxia due to erythroblastosis fetalis associated with hae moglobin H disease Acute massive myocardial infarction in the neonatal period is rare and usually is associated with congenital malformation of the heart or its blood supply Neonatal myocardial infarction in the anatomically normal heart with normal coronary vessels has been described in only 8 patients (1) This communication describes a newborn with acute haemorrhagic myocardial infarction due to anoxia believed to be caused by the combined effect of erythroblastosis fetalis and haemoglobin H disease

KEY WORDS Neonatal myocardial infarction Haemoglobin H Haemoglobin Bart s anoxia erythroblastosis

CLINICAL HISTORY

A male infant was delivered normally after 40 weeks of uneventful gestation The young parents second cousins are of Kurdish origin One male sibling 4 years old suffers from haemolytic anaemia secondary to haemoglobin H disease The birthweight was 3 130 kg The one minute Apgar score was 8 Within 30 minutes the infant developed progressive bradycardia bradypnea and cyanosis Rales were present over both lung fields The spleen and liver were grossly enlarged The electrocardiogram at the age of hours showed a pattern of myocardial injury with anterolateral wall and septal involvement as indicated by the prominent elevation of the S–T segment in leads V_1 V_2 V with a mirror S–T depression in L_3 V and AVR No distinct Q waves were seen (Fig 1)

There was a marked enlargement of the cardiac shadow at X-ray Cord blood haemoglobin electrophoresis showed that 43 % was haemoglobin Bart s The infant died at the age of 9 hours

The gross autopsy exhibited extensive petechial haemorrhages in the heart lungs thymus adrenals right kidney leptomeninges and brain The heart weighed 33 g (normal mean 20 1 g) with anatomically normal major blood vessels and valves The left ventricular wall disclosed a wide area of haemorrhagic discolouration Similar but less extensive changes were noticed in the right ventricle The spleen weighed 22 g (normal mean 9 7 g)

Microscopically sections of the heart revealed extensive haemorrhagic lakes which distorted and dissected between myocardial fibres The haemorrhagic lakes were rich in erythroblasts (Fig 2) Extensive extramedullary haematopoiesis with prominent erythroblastosis was observed in myocardium liver spleen kidneys adrenals and brain Practically all blood vessels contained excessive numbers of erythroblasts

DISCUSSION

Massive myocardial infarction in the newborn with an anatomically normal heart and coronary vessels is extremely rare It has been

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CASE REPORT

HAEMORRHAGIC INFARCTION OF THE MYOCARDIUM IN A NEWBORN WITH HAEMOGLOBIN H DISEASE AND ERYTHROBLASTOSIS

[redacted] GADOTH P KORNMEHL M GUERON and S W MOSES

From the Department of Paediatrics and the Cardiac Laboratory ¹ The Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheba Israel

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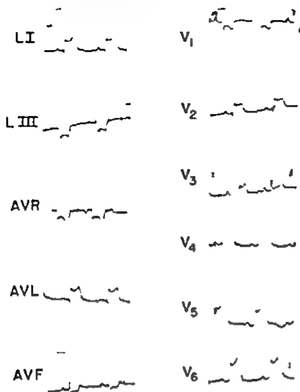


Fig 1 E C G at the age of 2 hours showing S–T elevation in $V_4 V_5 V_6$ with mirror depression in $L_3 V_1$ AVR

observed as a result of trauma embolism and rupture of papillary muscles (1 3) Neonatal myocardial infarction is associated with erythroblastosis fetalis but only rarely Bohr (4) mentions two such patients in whom focal areas of infarcted myocardium were d onstrated

This newborn suffered from haemoglobi disease He had a brother with haemoglobi disease and died from severe erythroblast fetalis with an excessive amount of B haemoglobin in his cord blood

Haemoglobin H disease is believed to double heterozygous state for alpha thal mia and haemoglobin H (5) Usually it ca haemolytic anaemia due to partial rep ment of the normal human haemoglobin the unstable haemoglobin H In the neo period it might cause hyperbilirubinaemia in reports from Malaya Singapore Hon and Thai hydrops fetalis was found (6)

The demonstration of increased amou haemoglobin Bart s in cord blood is ess for the diagnosis Haemoglobin H and ha globin Bart s both have abnormal oxyge sociation curves and lack the Bohr e whereby the release of oxygen to tiss markedly reduced (2) It is conceivable t this newborn with a large proporti haemoglobin Bart s in the circulating b the myocardial infarction occurred as sult of the severe non immune erythrob sis fetalis caused by haemoglobin H di

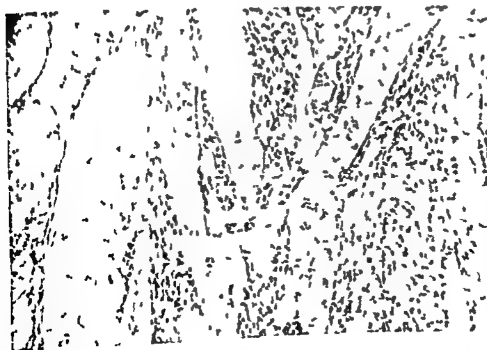


Fig 2 Left ventricular w (×135) There are essent lakes of blood separat myocardial fibres The majority of nucleated cel are erythroblasts Myocardial fibres are still nucleated and fairly well preserved

The relative inability of haemoglobin Bart s to release oxygen to tissues together with the haemorrhage are probably the causative factors for the infarction The lack of myocardial fibre necrosis may be a function of time since the myocardial lesion most probably developed after birth This concept is supported by the normal appearance at birth and the one minute Apgar score of 8

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CASE REPORT

MALIGNANT HISTIOCYTOSIS

Histiocytic Medullary Reticulosis

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ABSTRACT Ruuskanen O Kero [redacted] Rajamaki A Ekfors T Vilkki P and Nordman E (Departments of Paediatrics Haematology Pathology and Radiotherapy University of Turku Turku Finland) Malignant histiocytosis Histiocytic medullary reticulosis Acta Paediatr Scand 66 249 1977.—Three cases of malignant histiocytosis occurring in children aged 2 months [redacted] months and 14 years are described In all children the diagnosis was based on anaemia granulocytopenia or thrombocytopenia splenomegaly and marked erythrophagocytosis by bone marrow and lymph node atypical histiocytes Two children aged 10 months and 14 years underwent splenectomy after which combined chemotherapy with cyclophosphamide vincristine and prednisone (COP) was started In the older child a complete remission was achieved The younger child died soon after the onset of the treatment The youngest child was treated with bleomycin adriamycin cyclophosphamide vincristine and prednisone (BACOP) He died of pneumonia and sepsis two months after the start of the treatment

KEY WORDS Malignant histiocytosis combined chemotherapy

Malignant histiocytosis is a rare disease which is characterized by a systemic neoplastic proliferation of histiocytes and their precursors as described by Byrne & Rappaport (3) Clinical findings ususally consist of fever lymphadenopathy hepatosplenomegaly and preterminal jaundice Anemia leukopenia and thrombocytopenia are common laboratory findings (19) The diagnosis is difficult and the disease is usually rapidly fatal Recently however combined chemotherapy has given promising results and two complete remissions have been reported (2 18) In this paper we describe three children with malignant histiocytosis who were treated with combined chemotherapy In one of the children a complete remission was achieved lasting now over one year

CASE HISTORIES

Case 1

A [redacted] year-old girl was admitted to the local area hospital on July 1975 acutely ill with fever cough weakness stomatitis and infectious eczema behind the ears There was no lymphadenopathy hepatomegaly or splenomegaly Erythrocyte sedimentation rate (ESR) was 143 hemoglobin 58 g/l leukocytes 5 7×10⁹/l consisting of bands 11% granulocytes 44% eosinophils 14% lymphocytes 27% monocytes 5% and atypical cells 3% The amount of thrombocytes was 250×10⁹/l Bone marrow aspiration sample revealed erythroid hyperplasia with no specific changes Direct Coombs test was negative and serum bilirubin was normal The disease was considered *to be an atypical autoimmune hemolytic anemia.* The therapy included two units of packed red cells clindamycin and prednisolone After a 10-day hospitalization the girl did well and she was discharged ESR was 40 and Hb 97 g/l Prednisolone treatment was continued with a dose of 15 mg+15 mg+10 mg

In November 1975 she was admitted to the hospital because of fever and weakness ESR was 160 Hb 37 [redacted] and leukocytes 7 4×10⁹/l The girl was referred to Turku

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CASE REPORT

MALIGNANT HISTIOCYTOSIS

Histiocytic Medullary Reticulosis

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ABSTRACT Ruuskanen O Kero P Rajamaki A Ekfors T Vilkki P and Nordman E (Departments of Paediatrics Haematology Pathology and Radiotherapy University of Turku Turku Finland) Malignant histiocytosis Histiocytic medullary reticulosis Acta Paediatr Scand 66 249 1977.—Three cases of malignant histiocytosis occurring in children aged 2 months 10 months and 14 years are described In all children the diagnosis was based on anaemia granulocytopenia or thrombocytopenia splenomegaly and marked erythrophagocytosis by bone marrow and lymph node atypical histiocytes Two children aged 10 months and 14 years underwent splenectomy after which combined chemotherapy with cyclophosphamide vincristine and prednisone (COP) was started In the older child a complete remission was achieved The younger child died soon after the onset of the treatment The youngest child was treated with bleomycin adriamycin cyclophosphamide vincristine and prednisone (BACOP) He died of pneumonia and sepsis two months after the start of the treatment

KEY WORDS Malignant histiocytosis combined chemotherapy

Malignant histiocytosis is a rare disease which is characterized by a systemic neoplastic proliferation of histiocytes and their precursors as described by Byrne & Rappaport (3) Clinical findings ususally consist of fever lymphadenopathy hepatosplenomegaly and preterminal jaundice Anemia leukopenia and thrombocytopenia are common laboratory findings (19) The diagnosis is difficult and the disease is usually rapidly fatal Recently however combined chemotherapy has given promising results and two complete remissions have been reported (2 18) In this paper we describe three children with malignant histiocytosis who were treated with combined chemotherapy In one of the children a complete remission was achieved lasting now over one year

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Case 1

A 11 year-old girl was admitted to the local area hospital on July 1975 acutely ill with fever cough weakness stomatitis and infectious eczema behind the ears There was no lymphadenopathy hepatomegaly or splenomegaly Erythrocyte sedimentation rate (ESR) was 143 hemoglobin 58 g/l leukocytes 5 7×10⁹/l consisting of bands 11% granulocytes 44% eosinophils 14% lymphocytes 22% monocytes 5% and atypical cells 3% The amount of thrombocytes was 250×10⁹/l Bone marrow aspiration sample revealed erythroid hyperplasia with no specific changes Direct Coombs test was negative and serum bilirubin was normal The disease was considered to be an atypical autoimmune hemolytic anemia The therapy included two units of packed red cells clindamycin and prednisolone After a 10-day hospitalization the girl did well and she was discharged ESR was 40 and Hb 97 g/l Prednisolone treatment was continued with a dose of 15 mg+15 mg+10 mg

In November 1975 she was admitted to the hospital because of fever and weakness ESR was 160 Hb 32 g/l and leukocytes 4×10⁹/l The girl was referred to Turku





Figs 1 and 2 Malignant histiocyte in the bone marrow aspiration (1) and in the splenic sinus (2) of a 10-month old boy Note the numerous erythrocytes and a lymphocyte inside both cells ×1050

Fig 3 Malignant histiocyte showing erythrophagocytosis in the lymph node of a 2 month old boy ×1050

Fig 4 Malignant histiocyte in the bone marrow sample of a 14 year old girl Pathological erythrophagocytosis can be seen ×1050

University Central Hospital Physical examination revealed no lymphadenopathy splenomegaly or jaundice The patient was afebrile The number of leukocytes was 3.7×10^9/l consisting of 4% granulocytes 94% lymphocytes and 1% monocytes The number of thrombocytes was normal No reticulocytosis was found In addition the following studies were negative or normal direct Coombs test three bone marrow aspirations X ray films of chest skull plain film of abdomen long bones serum virus antibody examinations daily urinary excretions of methoxyhydroxymandelic acid (VMA) serum immunoglobulins NBT test coagulation studies repeated urine and stool tests for blood hemoglobin in plasma serum bilirubin urine urobilin Serum lactate dehydrogenase was 1310 U/l (normal <550 U/l) alkaline phosphatase 380 U/l (normal <970 U/l) glutamic oxalacetic transaminase 138 U/l (normal <35 U/l) Serum haptoglobin was low 0 15 g/l Using the whole blood micromethod the lymphocyte response to PHA was depressed and the response to ConA was within normal limits No response to PPD was found The proportional amount of E rosette forming lymphocytes (T lympho-

cytes) was 83%. The patient developed progressive anemia, the hemoglobin decreased 15–20 g/l in 2–3 days and during 30 days she received 13 units of packed red cells. Repeated re-examination of bone marrow aspirates revealed malignant histiocytes (Fig. 4) with erythrophagocytosis. The disease was considered to be malignant histiocytosis. Because of rapidly progressive anemia and granulocytopenia splenectomy was performed in December 1975. Samples were taken from hepatic and splenic hilar lymph nodes. An enlarged spleen weighing 500 g was found. A microscopic examination of the spleen showed normal basic structure with no atypical cells. Numerous histiocytes containing hemosiderin were found. The lymph node also had a normal structure, but both marginal and medullary sinuses contained histiocytes with erythrophagocytosis and leukophagocytosis. Some of these were in mitosis, but cytological atypia was not marked. The histological picture of the liver was normal.

The differential white blood cell counts two days before and two days after splenectomy were as follows:

	Leukocytes	Bands	Granulocytes	Eosinophils	Lymphocytes	Monocytes
Before	4.4×10⁹/l	1%	9%	5%	75%	7%
After	17.?×10⁹/l	3%	63%	–	30%	1%

After the operation the patient received cyclophosphamide 3 mg/kg for five consecutive days. Anemia continued but its progress was markedly limited. Three weeks after the operation COP inductions were started including cyclophosphamide 800 mg/m² i.v., vincristine 2 mg/m² i.v. and prednisone 60 mg/m² orally. Prednisone was given for five days and was then stopped gradually over five days. The patient received six COP inductions with two week intervals. Bone marrow examination after this initial treatment revealed no evidence of malignant histiocytosis. During the following year she received COP inductions ten times and felt perfectly well. The blood values were continuously normal. The only side-effect of the treatment was loss of hair. In June 1976 microscopic examination of axillary lymph nodes showed proliferation of atypical cells. The bone marrow sample was normal. A control lymph node examination in December 1976 revealed normal findings. The patient is considered to be in complete remission and has remained well. The COP inductions will be given every two months during the following year.

Case 2

A 10-month-old boy was admitted to the local area hospital in May 1976 because of bronchopneumonia. The laboratory values were as follows: ESR 22, hemoglobin [illegible] g/l, leukocytes 4.0×10⁹/l with 3% bands, 11% granulocytes, 68% lymphocytes and 18% monocytes. The platelet count was 90×10⁹/l. Bone marrow aspiration revealed probable iron deficiency anemia with no evidence of malignancy. The child was treated with azidocillin and he was discharged in good condition. A septic type of fever, however, started after two weeks and because of anemia and pancytopenia the child was referred to Turku University Central Hospital.

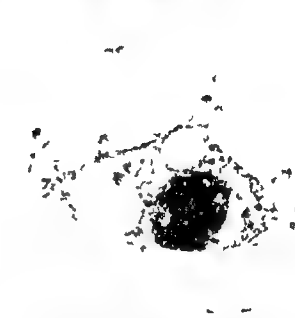
On physical examination he appeared chronically ill and hepatosplenomegaly was found. The hematologic findings were as follows: ESR 40, hemoglobin 60 g/l, white blood cell count 2.8×10⁹/l with 21% bands, 32% granulocytes, 1% basophils, 39% lymphocytes and 6% monocytes. A bone marrow aspiration showed effective erythropoiesis with no malignant cells. Direct Coombs's test was negative. The platelet count was 30×10⁹/l. The following laboratory studies were negative or normal: X-ray films of chest, long bones and skull, i.v. urography and renal angiography, repeated blood, urine and cerebrospinal fluid cultures, tests for tuberculosis, syphilis, malaria, tularemia, toxoplasmosis, salmonellosis, numerous virus antibodies in serum, serum muramidase, serum acid phosphatase, NBT test, serum immunoglobulins, serum cholesterol, serum triglycerides, blood coagulation studies, daily urinary excretions of VMA, serum haptoglobin, serum lactate dehydrogenase, alkaline phosphatase and serum glutamic oxalacetic transaminase. The white blood cell differential count revealed a progressive granulocytopenia with relative lymphocytosis. The lymphocyte responses to PHA and ConA were depressed. The clinical findings were characterized by temperature spikes from a baseline to 40.0°C. The child was treated with ampicillin, digoxin, penicillin+gentamycin and prednisolone+streptomycin with no effect. Due to progressive anemia, thrombocytopenia and granulocytopenia splenectomy was performed four weeks after admission to the hospital and the amount of leukocytes and the proportional amount of granulocytes rose and anemia was corrected. The spleen weighed 490 g. On microscopic examination a normal basic structure was found. Both sinusoids and red pulp cords contained numerous histiocytes packed with erythrocytes. At this stage this finding was interpreted as a reactive change. Lymph nodes from splenic hilus showed necrotic changes suggesting active tuberculosis. An experimental combination treatment with rifampicin, streptomycin and isoniazid for ten days yielded, however, no improvement. The general condition of the patient became gradually worse. The blood coagulation studies and serum lactic dehydrogenase, alkaline phosphatase and serum glutamic oxalacetic transaminase changed to pathologic values. The child became icteric, serum bilirubin rose to 69 µmol/l. A new cervical lymph node sample contained malignant histiocytes and the third bone marrow aspiration revealed histiocytes with intense erythrophagocytosis. Histiocytes containing thrombocytes, lymphocytes and granulocytes were also found (Figs. 1 and 2). Re-examination of the splenic sample and the previous bone marrow aspirations showed similar cells, which were also now found in the cerebrospinal fluid. On the basis of the clinical and histological findings the disease was considered to be malignant histiocytosis. COP treatment with the same regimen as in case 1 was started. After two COP inductions no improvement was found and the child died three weeks after the start of the chemotherapy and after 2.5 months treatment in the hospital.

At autopsy liver was enlarged weighing 670 g. Also lymph nodes were macroscopically enlarged. The lower part of the oesophagus, cardia of the stomach and the









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On physical examination he appeared chronically ill and hepatosplenomegaly was found. The hematologic findings were as follows: ESR 40, hemoglobin 60 g/l, white blood cell count 2.8×10^9/l with 21% bands, 32% granulocytes, 1% basophils, 39% lymphocytes and 6% monocytes. A bone marrow aspiration showed effective erythropoiesis with no malignant cells. Direct Coombs's test was negative. The platelet count was 30×10^9/l. The following laboratory studies were negative or normal: X-ray films of chest, long bones and skull, i.v. urography and renal angiography, repeated blood, urine and cerebrospinal fluid cultures, tests for tuberculosis, syphilis, malaria, tularemia, toxoplasmosis, salmonellosis, numerous virus antibodies in serum, serum muramidase, serum acid phosphatase, NBT test, serum immunoglobulins, serum cholesterol, serum triglycerides, blood coagulation studies, daily urinary excretions of VMA, serum haptoglobin, serum lactate dehydrogenase, alkaline phosphatase and serum glutamic oxalacetic transaminase. The white blood cell differential count revealed a progressive granulocytopenia with relative lymphocytosis. The lymphocyte responses to PHA and ConA were depressed. The clinical findings were characterized by temperature spikes from a baseline to 40.0°C. The child was treated with ampicillin, digoxin, penicillin+gentamycin and prednisolone+streptomycin with no effect. Due to progressive anemia, thrombocytopenia and granulocytopenia splenectomy was performed four weeks after admission to the hospital and the amount of leukocytes and the proportional amount of granulocytes rose and anemia was corrected. The spleen weighed 490 g. On microscopic examination a normal basic structure was found. Both sinusoids and red pulp cords contained numerous histiocytes packed with erythrocytes. At this stage this finding was interpreted as a reactive change. Lymph nodes from splenic hilus showed necrotic changes suggesting areactive tuberculosis. An experimental combination treatment with rifampicin, streptomycin and isoniazid for ten days yielded, however, no improvement. The general condition of the patient became gradually worse. The blood coagulation studies and serum lactic dehydrogenase, alkaline phosphatase and serum glutamic oxalacetic transaminase changed to pathologic values. The child became icteric, serum bilirubin rose to 69 μmol/l. A new cervical lymph node sample contained malignant histiocytes and the third bone marrow aspiration revealed histiocytes with intense erythrophagocytosis. Histiocytes containing thrombocytes, lymphocytes and granulocytes were also found (Figs. 1 and 2). Re-examination of the splenic sample and the previous bone marrow aspirations showed similar cells which were also now found in the cerebrospinal fluid. On the basis of the clinical and histological findings the disease was considered to be malignant histiocytosis. COP treatment with the same regimen as in case 1 was started. After two COP inductions no improvement was found and the child died three weeks after the start of the chemotherapy and after 2.5 months treatment in the hospital.

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Malignant histiocytosis has been considered to be a rare disease Only over 100 cases have been reported in the literature under a variety of names (1 19) The rarity might be partly due to the difficult diagnosis and new case reports are currently published (4 12 17) Our observations support the view that combination chemotherapy will markedly improve the prognosis of this disease

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colon showed extensive candidiasis Microscopically lymph nodes liver and kidney contained atypical histiocytes with erythrophagocytosis and leukophagocytosis

Case 3

A 2 month old boy was referred to Turku University Central Hospital in January 1977 because of thrombocytopenia and diarrhea On physical examination he appeared chronically ill with a scally erythematous papular rash on the neck trunk and upper arms Hepatosplenomegaly and cervical lymphadenopathy were found The laboratory values were as follows ESR 35 hemoglobin 124 g/l leucocytes 10 2×10⁹/l with 5% bands 30% granulocytes 55% lymphocytes and 10% monocytes The platelet count was 20×10⁹/l Bone marrow aspiration revealed effective erythropoiesis and normal megacaryocytic count with many immunoblasts No evidence of malignancy was found Radiographic examination of the chest revealed mild pulmonary infiltrations and the patient was treated with penicillin A septic type of fever started and continuous gastrointestinal bleeding and anemia developed The following laboratory and physical studies were negative or normal X ray films of long bones and skull repeated blood urine and cerebrospinal fluid cultures tests for tuberculosis syphilis salmonellosis numerous virus antibodies (including EB titers) blood coagulation studies alkaline phosphatase serum glutamic oxalacetic transaminase serum muramidase serum immunoglobulins serum cholesterol plasma triglycerides and daily urinary VMA The lymphocyte response to PHA and ConA were normal Serum lactic dehydrogenase was pathologic 1090 U/l (normal <440 U/l) Skin biopsy revealed subacute dermatitis with no malignant cells In the lymph node biopsy atypical histiocytes with marked erythrophagocytosis were found within the subcapsular and medullary sinuses (Fig 3) There were also numerous eosinophils The second bone marrow aspiration two weeks after admission revealed histiocytes with erythrophagocytosis On the basis of the clinical and histological findings the disease was considered to be malignant histiocytosis

A combination chemotherapy program consisting of bleomycin adriamycin cyclophosphamide vincristine and prednisone (BACOP) was started according to Schein et al (15) Adriamycin (25 mg/m²) cyclophosphamide (650 mg/m²) and vincristine (1 4 mg/m²) were administered intravenously on days 1 and 8 This was followed by a nonmyelosuppressive phase with bleomycin (5 mg/m²) and prednisone (60 mg/m²) orally on days 15 through 29 The child received two cycles of BACOP inductions During the second cycle Staphylococcus aureus sepsis developed which was treated with cephalosporin This was followed by E coli pneumonia and sepsis and the child died of heart failure pulmonary oedema and hyponatremia

At autopsy enlarged liver and spleen weighing 280 g and 25 g respectively were found No enlarged lymph nodes were discovered The thymus showed marked lymphocyte depletion In one lymph node there were scantily atypical histiocytes No atypical histiocytes could be found in bone marrow liver and spleen Severe candidiasis was found in the oesophagus The lungs showed severe pneumonic changes and infection was considered to be the cause of death

DISCUSSION

The diagnosis of malignant histiocytosis was based in all of our patients on appearance of numerous atypical histiocytes with erythrophagocytosis in the lymph nodes and/or bone marrow Byrne & Rappaport (3) have considered these cells to be an essential criterion for the diagnosis Anemia granulocytopenia thrombocytopenia and splenomegaly are also typical features of this disease which has also been called histiocytic medullary reticulosis by Scott & Robb Smith (16) Abundant eosinophilia or eosinophilic reaction in the lymph node was observed in two of the patients Henderson & Sage (9) have described eosinophilia in one patient with malignant histiocytosis

The diagnosis of malignant histiocytosis may be difficult and is often made in the terminal phase of the disease Elevated serum muramidase and acid phosphatase activities have been found in single cases and have been suggested to be of diagnostic value (7 10 17 20) These enzyme activities were measured in cases 2 and 3 but were found to be within normal limits Merrill & Barrett (13) found a positive monospot test in a patient with histiocytic medullary reticulosis and suggested that Epstein Barr virus may play some role in the etiology of this disease No EB antibodies were found in any of our patients

The differentiation of malignant histiocytosis from Letterer Siwe disease is difficult The occurrence of cytological atypia and the lack of tumor nodules justifies the diagnosis in our cases (3 5 12) Furthermore abundant erythrophagocytosis is a feature of malignant histiocytosis rather than Letterer Siwe disease (17) The findings in the two young children were similar to those in congenital hemophagocytic reticulosis a disease which has been suggested to be an infantile form of malignant histiocytosis (11) In all the entity of histiocytic disorders is confus

ing and poorly understood and needs a sim plified classification as suggested by Cline & Golde (5)

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Case 3

A 2 month old boy was referred to Turku University Central Hospital in January 1977 because of thrombocytopenia and diarrhea On physical examination he appeared chronically ill with a scally erythematous papular rash on the neck trunk and upper arms Hepatosplenomegaly and cervical lymphadenopathy were found The laboratory values were as follows ESR 35 hemoglobin 124 g/l leucocytes 10 2×10⁹/l with 5% bands 30% granulocytes 55% lymphocytes and 10% monocytes The platelet count was 20×10⁹/l Bone marrow aspiration revealed effective erythropoiesis and normal megacaryocytic count with many immunoblasts No evidence of malignancy was found Radiographic examination of the chest revealed mild pulmonary infiltrations and the patient was treated with penicillin A septic type of fever started and continuous gastrointestinal bleeding and anemia developed The following laboratory and physical studies were negative or normal X ray films of long bones and skull repeated blood urine and cerebrospinal fluid cultures tests for tuberculosis syphilis salmonellosis numerous virus antibodies (including EB titers) blood coagulation studies alkaline phosphatase serum glutamic oxalacetic transaminase serum muramidase serum immunoglobulins serum cholesterol plasma triglycerides and daily urinary VMA The lymphocyte response to PHA and ConA were normal Serum lactic dehydrogenase was pathologic 1090 U/l (normal <440 U/l) Skin biopsy revealed subacute dermatitis with no malignant cells In the lymph node biopsy atypical histiocytes with marked erythrophagocytosis were found within the subcapsular and medullary sinuses (Fig 3) There were also numerous eosinophils The second bone marrow aspiration two weeks after admission revealed histiocytes with erythrophagocytosis On the basis of the clinical and histological findings the disease was considered to be malignant histiocytosis

A combination chemotherapy program consisting of bleomycin adriamycin cyclophosphamide vincristine and prednisone (BACOP) was started according to Schein et al (15) Adriamycin (25 mg/m²) cyclophosphamide (650 mg/m²) and vincristine (1 4 mg/m²) were administered intravenously on days 1 and 8 This was followed by a nonmyelosuppressive phase with bleomycin (5 mg/m²) and prednisone (60 mg/m²) orally on days 15 through 29 The child received two cycles of BACOP inductions During the second cycle Staphylococcus aureus sepsis developed which was treated with cephalosporin This was followed by E coli pneumonia and sepsis and the child died of heart failure pulmonary oedema and hyponatremia

At autopsy enlarged liver and spleen weighing 280 g and 25 g respectively were found No enlarged lymph nodes were discovered The thymus showed marked lymphocyte depletion In one lymph node there were scantily atypical histiocytes No atypical histiocytes could be found in bone marrow liver and spleen Severe candidiasis was found in the oesophagus The lungs showed severe pneumonic changes and infection was considered to be the cause of death

DISCUSSION

The diagnosis of malignant histiocytosis was based in all of our patients on appearance of numerous atypical histiocytes with erythrophagocytosis in the lymph nodes and/or bone marrow Byrne & Rappaport (3) have considered these cells to be an essential criterion for the diagnosis Anemia granulocytopenia thrombocytopenia and splenomegaly are also typical features of this disease which has also been called histiocytic medullary reticulosis by Scott & Robb Smith (16) Abundant eosinophilia or eosinophilic reaction in the lymph node was observed in two of the patients Henderson & Sage (9) have described eosinophilia in one patient with malignant histiocytosis

The diagnosis of malignant histiocytosis may be difficult and is often made in the terminal phase of the disease Elevated serum muramidase and acid phosphatase activities have been found in single cases and have been suggested to be of diagnostic value (7 10 17 20) These enzyme activities were measured in cases 2 and 3 but were found to be within normal limits Merrill & Barrett (13) found a positive monospot test in a patient with histiocytic medullary reticulosis and suggested that Epstein Barr virus may play some role in the etiology of this disease No EB antibodies were found in any of our patients

The differentiation of malignant histiocytosis from Letterer Siwe disease is difficult The occurrence of cytological atypia and the lack of tumor nodules justifies the diagnosis in our cases (3 5 12) Furthermore abundant erythrophagocytosis is a feature of malignant histiocytosis rather than Letterer Siwe disease (17) The findings in the two young children were similar to those in congenital hemophagocytic reticulosis a disease which has been suggested to be an infantile form of malignant histiocytosis (11) In all, the entity of histiocytic disorders is confus

CASE REPORT

PSEUDOHYPOALDOSTERONISM

Clinical Biochemical and Morphological Studies in a Long term Follow up

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KEY WORDS Pseudohypoaldosteronism salt losing syndrome renal tubular acidosis aldosterone renin malnutrition

Since the first description by Cheek & Perry in 1958 of an unusual salt wasting syndrome in infancy a total of at least [illegible] cases have been described (1 13 17 18 20 21) The common characteristics of these infants are failure to thrive anorexia vomiting dehydration urinary sodium loss hyponatraemia and hyperkalaemia Overall renal function and adrenal cortisol/corticosterone secretion is normal Laboratory studies have documented an increased rate of aldosterone secretion and the term pseudohypoaldosteronism has been coined This designation implies an apparent insensitivity of the renal tubule to high levels of endogenous aldosterone as a crucial pathogenetic factor However there is no consensus of opinion as far as the pathophysiology of the syndrome is concerned

CASE HISTORY

A male infant was born in 1970 after an uncomplicated pregnancy as the first child of healthy unrelated parents Birth weight 2700 g length 47 cm At the age of 6 days the infant was admitted to the neonatal ward with jaundice Apart from jaundice physical examination revealed no abnormalities the external genitals were normal Whereas hyperbilirubinemia disappeared within a few days the patient remained drowsy with regurgitation and vomiting Self-evident dehydration was not apparent No signs of infectious disease were disclosed and the urine was free of

19 Warnke R A Kim H & Dorfman R F Malignant histiocytosis (Histiocytic medullary reticulosis) *Cancer 35* 215 1975
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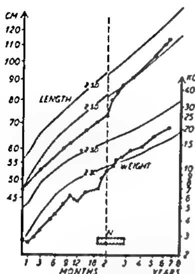


Fig 1 Growth curve of the patient in relation to the normal material of Karlberg et al (12) Hatching shows the period in which supplementary sodium was given

glucose protein and amino acids Intravenous pyelography and micturition cysto-urethrography were normal Weight gain was very slow (Fig 1)

The infant was readmitted because of vomiting constipation and dehydration at the age of 3 months After rehydration food intake became normal and vomiting ceased The child was hypotonic with retarded physical development but mental development was apparently normal

At the age of 10 months X ray studies showed halisteresis and retarded bone age At 12 months the weight was 5 520 g and dentition was absent

Pseudohypoaldosteronism was diagnosed The patient was treated with sodium bicarbonate 24 mmol per day over a period of 10 months from the age of 19 months and subsequently with sodium chloride 26 mmol per day for 2 months During and after this treatment catch up growth was evident (Fig 1) The general condition was excellent with normal physical and mental development Halisteresis disappeared and the bone age caught up with the chronological age

The child was followed closely up to the age of 7 years Both physical and mental development remained normal Hyponatraemia and hyperkalaemia did not recur the blood pressure remained normal and a normal glomerular filtration rate was attained

METHODS

Plasma renin

Plasma renin concentration was measured according to Giese et al (10) Appropriate modifications of the analytical technique were made in order to determine the extremely high plasma renin levels encountered in samples drawn during the initial phase of the study

Adrenal function

Plasma aldosterone concentration was measured by a modification of the method of Mayes et al tion of free and antibody bound aldosterone was performed by gel centrifugation

Urinary excretion of tetrahydroaldosterone was measured as described by Damkjær Nielsen et al (5) Urinary excretion of corticosteroid metabolites was measured as described by Damkjær Nielsen et al (4)

Renal function

Glomerular filtration rate was evaluated by measurements of creatinine clearance or ^{51}Cr EDTA clearance (?)

Renal morphology

A surgical biopsy from the right kidney was obtained when the patient was 19 months old The tissue was fixed in Lillie s buffered formaldehyde After processing ? µm sections were stained with periodic acid–silver methen amine

Homeostatic response to low sodium intake

At the age of 6$^{8}/_{12}$ years the following test was carried out After collection of pre test samples the child received a low sodium diet (10 mmol/day) for a period of 6 days Plasma electrolytes urinary electrolyte losses and the parameters of the renin–aldosterone system were monitored

Acid–base homeostasis

Ammonium chloride loading was performed according to Wrong & Davies (23) Urine pH was measured immediately after voiding Net acid excretion was measured by the method of Jørgensen (11) These parameters were followed for 5 hours after administration of ammonium chloride which was given by mouth for the first test and by intravenous infusion of isotonic ammonium chloride for the later examinations Details concerning dosage and times of examination are given in Table 3 All tests were done with the patient on an ordinary diet sodium chloride or sodium bicarbonate was not given at the time of the tests

Sweat chloride concentration

Quantitative sweat test was performed by iontophoresis of pilocarpin (9)

Plasma volume

Determination of plasma volume was carried out by isotope dilution (^{131}I albumin)

Calcium studies

Serum calcium (total and ultrafiltrable) serum phosphorus and serum alkaline phosphatase were determined together with the urinary excretion and the tubular reabsorption rate of calcium over a 5 day period on a low calcium diet at the age of 12 months

RESULTS

Renin–aldosterone system

The results of long term studies of electrolyte

Table 1 *Plasma electrolytes renin and aldosterone concentrations*

Age (years)	Se Na (mmol/l)	Se K (mmol/l)	Plasma renin concentration (mIU/l)	Plasma aldosterone concentration (nmol/l)	Urinary tetrahydro-aldosterone (nmol/d)	Treatment
0 /	1?8	6 6				0
0 /	130	5 0				[illegible]
1 /	1?4	5 3	70 000	3 3	1 155	0
1 /	1?3	4 8	4 ?00			0
? /	139	4 6	375	5 3		$NaHCO_3$ 24 mmol/d
? /	14?	4 3	325	8 ?		NaHCO 24 mmol/d
? /	141	4 1	?63	9 5		NaCl ?6 mmol/d
? /	138	4 7	4 0	10 6	?34	0
? /			560	[illegible] 1	600	[illegible]
3 /					1 034	0
3 /	143	4 8			3?7	0
3 /					836	0
3 /	135		1 000	12 0	1 359	0
3 /					1 207	0
3 /	136	4 4	1 300	14 9		0
4 /					891	0
4 /	143	3 8	367	3 4		0
5 /	136	4 0	1 400	11 4		0
6 /	143	4 5	310	12 7		0
6 /	134	4 3	5 900	27 5	? ?44	Na depletion
6 /					836	0
Normal ranges (1–3 years)	132–144	3 6–4 8	?5–312	0 3–? 4	<80	

aldosterone concentration and urinary tetrahydroaldosterone excretion rate are given in Table 1 Before treatment was started hyponatraemia was a prominent laboratory finding At this stage the plasma renin concentration was excessively high—in fact this is the highest value ever measured in our laboratories At the same time aldosterone parameters were indicative of a pronounced activation of aldosterone secretion

Treatment with sodium bicarbonate rapidly restored normal serum sodium levels and plasma renin concentration was promptly reduced to moderately elevated values However plasma aldosterone concentration and urinary tetrahydroaldosterone excretion rate remained definitely elevated This held true during the subsequent period of sodium chloride treatment

After the treatment was stopped at the age of 31 months repeated examinations over several years showed fairly normal serum sodium concentration elevated plasma renin concentration and plasma aldosterone concentration definitely increased urinary tetrahydroaldosterone excretion rate but a persistently normal serum potassium concentration

Adrenal cortisol and corticosterone biosynthesis

The urinary excretion of corticosteroid metabolites was measured at three different age levels as detailed in Table 2 On all occasions the differentiated metabolite excretion pattern did not deviate from that observed in normal children matched for age

Glomerular filtration rate

The following values were obtained all given in ml/minute×1 73 m² (12 months) 40 (17 months) 65 ($4^1/_2$years) 92 and ($6^1/_2$years) 109

Renal biopsy

The specimen comprised tissue elements from cortex medulla and papilla A total of about 200 renal corpuscles were examined The glomerular tufts were essentially normal whereas a considerable hypertrophy of the

Table 2 *Urinary excretion of corticosteroid metabolites*

Total F Tetrahydrocortisol allo tetrahydrocortisol tetrahydrocortisone cortisol and cortisone
THS Tetrahydro-11 desoxycortisol
Total B Tetrahydrocorticosterone allo-tetrahydrocorticosterone tetra 11 dehydrocorticosterone corticosterone and 11-dehydrocorticosterone
THDOC Tetrahydrodesoxycorticosterone
P triol Pregnanetriol
Δ 5 P triol Pregnenetriol

Age (years)	Total F (μmol/d)	THS (μmol/d)	Total B (μmol/d)	THDOC (μmol/d)	P triol (μmol/d)	Δ 5 P triol (μmol/d)
0 11/12	2 2	0 06	0 77	0	0	0
2 11/12	0 5	0 02	0 11	0	0	0
3 8/12	1 6	0 09	0 54	0	0 03	0
Normal ranges 1–4 years	0 8–6 6	<0 23	0 26–1 28	0	<0 12	0

juxtaglomerular apparatus was a prominent feature (Fig 3) In almost all renal corpuscles a broad rim of epithelioid cells was found between the vas afferens and the macula densa These cells contained many silver stained granules The tubular system the collecting ducts, the interstitial tissue and the papilla were normal Blood vessels were normal apart from hyperplasia of the muscular coat of the arterioles approaching the juxtaglomerular apparatus

Response to low sodium intake

The following features were disclosed (Table 1) 1) A definite though not dramatic decrease in serum sodium concentration 2) a reduction of renal sodium excretion to a level of approximately 15 mmol/d 3) a pronounced activation of the renin system as reflected in a 20 fold increase of plasma renin concentration 4) a marked stimulation of aldosterone secretion with a doubling of the plasma aldosterone concentration The clinical observation during the test did not reveal signs of dehydration or other adverse effects

Acid–base homeostasis

During the first admission a slight partially compensated metabolic acidosis was noticed (capillary blood pH=7 32 P_{CO_2}=4 7 kPa (35 mmHg) standard HCO_3=18 mmol/l and base excess=−7 5 mmol/l) A compensated metabolic acidosis persisted during the second and third admission Spontaneous urine pH was high (6 0–8 0)

In Fig 2 the urinary pH response during ammonium chloride loading is depicted as a function of time Normal values are taken from Wrong & Davies (23)

The test performed at the age of 11 months was clearly abnormal since the minimal pH achieved was consistently above the normal range throughout the test In the subsequent tests the response was still abnormal with a sluggish fall in pH as compared with the normal response although a single urine specimen showed a pH value within the upper part of normal range

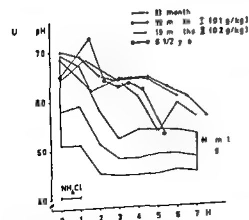


Fig 2 Urinary pH response to ammonium chloride loading performed at different ages Normal ranges and mean values are taken from Wrong & Davies (23) and are indicated by the solid lines without symbols

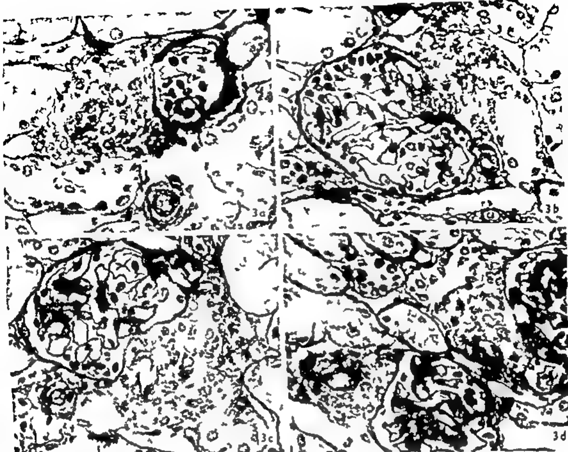


Fig 3 Renal biopsy specimen age 1⁷/₁₂ years showing extreme hyperplasia of the juxtaglomerular epithelioid cells Periodic acid–silver methenamine stain Polaroid film Original magnification ×250

Additional data are given in Table 3 The net acid excretion corresponds to the lower part of the reference interval (7 8)

Sweat test

Sweat chloride concentration was 52 mmol/l at the age of 1 year and 23 mmol/l at the age of 6¹/₂ years The first value is slightly raised the second quite normal

Plasma volume

Plasma volume was 39 ml/kg body weight at the age of 1⁵/₁₂ years The normal value is 52 ml/kg (16)

Table 3 *Ammonium chloride loading*

Age (years)	pH in urine		Net acid excretion (µmol/min 1 73 m)	Minimal standard bicarbonate (mmol/l)	Changes in base excess during load (mmol/l)	Dose of NH_4Cl	
	spontaneous	after loading				mmol/kg	g/kg
0 /	7 00	6 09–6 5?		17		1 9	0 1
1 /	6 85	5 67–6 4?	59–108	17	−6	1 9	0 1
1 /	6 43	5 33–6 [illegible]		13	−8	3 7	0 ?
6 /	6 48	5 30–7 40	.6– 88	21	−4	1 9	0 1

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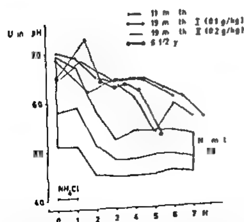


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small amounts of bicarbonate were sufficient to correct the acidosis

In the renal tubule the transport mechanisms for sodium potassium and protons are interrelated in a complex manner partly regulated by aldosterone Hence the presence of an urinary acidification defect in pseudohypoaldosteronism is not surprising However RTA is not a constant feature of pseudohypoaldosteronism

The response to a low sodium intake was studied in this case with an express purpose It would seem important to known whether the child enjoying normal health under normal circumstances would be able to respond to a situation taxing the mechanisms for sodium conservation It would indeed seem reasonable to take particular care in replacement of sodium losses in the event of gastrointestinal tract infections etc

Though rare the syndrome of pseudohypoaldosteronism represents a clinical challenge in particular because a treatment of utter simplicity may exert profound beneficial effects on a disease condition which in the absence of proper therapy can be quite dangerous or even fatal

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Calcium metabolism

Serum calcium serum phosphorus and al kaline phosphatase were normal for age The urinary excretion of calcium and the tubular reabsorption rate of calcium determined dur ing a 5 day period of low calcium intake were normal

DISCUSSION

Our patient presented several symptoms de scribed in previously reported cases of pseudo hypoaldosteronism Malnutrition and hypo natraemia lead to evaluation of adrenocortical function The normal excretion of pregnane triol throughout the study excludes the pos sibility of a 21 hydroxylation defect in steroid biosynthesis

The negligible excretion of Δ 5 pregnene triol excludes the very rare 3β ol dehydrogenase deficiency Congenital adrenocortical hypoplasia and 18 hydroxylase deficiency are excluded on the basis of normal cortisol secre tion and the increased aldosterone secretion respectively

The glomerular filtration rate was subnor mal over the first 17 months of the patient s life, whereas normal values were attained at a later stage

During the initial period of hyponatraemia an extreme activation of the renin–aldosterone system was demonstrated Even though the treatment with sodium bicarbonate restored the sodium level to normal values increased levels of renin and aldosterone persisted throughout the 7 years of observation indi cating a state of chronic hyperaldosteronism The data obtained during the sodium restric tion test performed at the age of 6⁸/₁₂ years showed, that the renin–aldosterone system could be activated much further indicating a considerable functional reserve capacity

Donnell et al (6) described a 100 fold in crease in urinary aldosterone excretion in their case with subsequent normalization after sodium supplementation High plasma renin activity was first described by Trung in 1970 (22) and this finding has later been confirmed by other authors

Cheek & Perry (3) in their original descrip tion of the first case of pseudohypoaldo steronism in 1958 focused upon an unrespon siveness of the distal renal tubule to aldo sterone as a major pathophysiological mech anism That the tubular receptors are not to tally insensitive to aldosterone is made pro bable by the finding that administration of aldo sterone antagonists induces an increased renal loss of sodium (19) Offhand the clinical course would make the hypothesis tenable that progressive tubular maturation or increase of sensitivity at the receptor level with increasing age accounts for the fact that the sodium re quirement seems to decline In previously re ported cases sodium supplementation could be withdrawn after some months without any ill effects (3 17) However as clearly docu mented by the biochemical findings in our case a fairly normal sodium homeostasis is achieved at the expense of a permanent activa tion of the renin–aldosterone system

Another hypothesis holds that the funda mental defect is a disturbance in proximal tubular sodium reabsorption leading to an excessive sodium load to the distal tubule with attendant sodium wasting and secondary hyperaldosteronism (18) Our observations provide no information permitting us to eval uate the relative merits of these two major hypotheses

The finding of a metabolic acidosis and an inappropriately high urinary pH suggest a form of renal tubular acidosis (RTA) The abnormal response of pH in urine to an acid load and the low net acid excretion support this assumption (Table 3 Fig 2)

A distinction is commonly drawn between a proximal and a distal form of RTA (15) In the present case the tubular defect may be classi fied as being of the distal type In spite of a low plasma bicarbonate the urinary pH did not de crease sufficiently during the third ammonium chloride test Bicarbonate loss in urine typical of the proximal type of RTA is unlikely since

small amounts of bicarbonate were sufficient to correct the acidosis

In the renal tubule the transport mechan isms for sodium potassium and protons are interrelated in a complex manner partly regu lated by aldosterone Hence the presence of an urinary acidification defect in pseudo hypoaldosteronism [redacted] not surprising How ever RTA is not a constant feature of pseudo-hypoaldosteronism

The response to a low sodium intake was studied in this case with an express purpose It would seem important to known whether the child enjoying normal health under normal circumstances would be able to respond to a situation taxing the mechanisms for sodium conservation It would indeed seem reason able to take particular care in replacement of sodium losses in the event of gastrointestinal tract infections etc

Though rare the syndrome of pseudohypo-aldosteronism represents a clinical challenge in particular because a treatment of utter simplicity may exert profound beneficial ef fects on a disease condition which in the ab sen e of proper therapy can be quite dan gerous or even fatal

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Denmark

ANNOUNCEMENTS

XIth MIDDLE EASTERN-MEDITERRANEAN PAEDIATRIC CONGRESS

The XIth Middle Eastern-Mediterranean Paediatric Congress will take place in Marseille France 4 5 6 September 1978 The scientific programme will comprise of plenary sessions symposia and free papers The programme will cover multiple disciplines of paediatrics President Pr Rene Bernard Secretary General Pr F Giraud Adress Hopital d Enfants de la Timone 13385 Marseille Cedex 4 France

6th EUROPEAN CONGRESS OF PERINATAL MEDICINE

The 6th European Congress of Perinatal Medicine will take place in Vienna Austria August 30th–September 1st 1978 Information may be obtained from the secretariat Interconvention Kinderspitalgasse 5 A 1095 Vienna Austria

NEW BOOKS RECEIVED

O Craig *Childhood diabetes and its management* 765 pp illus *In* J Apley (ed) Postgraduate paediatric series Butterworths London Boston 1977 £8 50 ISBN 0-40 -00176-3

Murray Davidson (ed) *Paediatric gastroenterology* 503 pp illus *In* Clinics in gastroenterology vol 6 no 2 W B Saunders Company Ltd London Philadelphia Toronto 1977 £15 00 Subscription (3 issues) £7 50 Single copy

M H Donaldson & G H Seydel (eds) *Trends in childhood cancer* 144 pp illus John Wiley & Sons New York London Sydney Toronto 1977 £12 40

I R S Gordon & I G M Ross *Diagnostic radiology in paediatrics* 384 pp illus *In* J Apley (ed) Postgraduate paediatrics series Butterworths London Boston 1977 £19 50 ISBN 0-407-00171

J Noble (ed) *Primary care and the practice of medicine* 360 pp illus Little Brown and Company (Inc) Boston 1976 No price given ISBN 0-316-61148-4

J S Remington & J O Klein (eds) *Infectious disease of the fetus and newborn infant* 1171 pp illus Holt Saunders Ltd Eastbourne 1976 £36 50

I Valadian & D Porter (eds) *Physical growth and development From conception to maturity A programmed text* 338 pp illus Little Brown and Company (Inc) Boston 1977 Price not given ISBN 0-316-89535 3

R G Williams & C R Tucker *Echocardiographic diagnosis of congenital heart disease* 352 pp illus Little Brown and Company (Inc) Boston 1977 US $19 95 ISBN 0-316-94351 7

J G Wilson & F C Fraser (eds) *Handbook of teratology* vol 1 General principles and etiology 476 pp illus vol 2 Mechanisms and pathogenesis 491 pp illus Plenum Press New York London 1977 Vol 1 ISBN 0-306-36241-4 US$54 00 Vol 2 ISBN 0-306-36242 2 US $46 00

R Landolt (ed) *Aktuelle Probleme der pädiatrischen Hepatologie* 68 pp illus *In* E Rossi (ed) Pädiatrische Fortbildungskurse für die Praxis S Karger Basel München Paris London New York Sydney 1977 DM 24 – ISBN 3 8055 2667 8

T Hirata & J P Brady *Newborn intensive care Chemical aspects* 179 pp illus Charles C Thomas Publisher Springfield 1977 US $16 75 ISBN 0-398-03606-3

J de Grouchy & C Turleau *Atlas des maladies chromosomiques* 356 pp illus Expansion Scientifique Paris 1977 Fr 273 50 ISBN 7046-0901 ?

S Krugman R Ward & S L Katz *Infectious diseases of children* 6th ed 539 pp illus The C V Mosby Company Saint Louis 1977 ISBN 0-8016-2800-8

J Strauss (ed) *Pediatric nephrology* vol 3 Current concepts in diagnosis and management 401 pp illus Plenum Press New York London 1976 US $35 40 ISBN 0-306-32303-6

Acta Pædiatr Scand 67 265–268 1978

HIGH DOSE METHOTREXATE IN ACUTE LYMPHOCYTIC LEUKEMIA IN CHILDHOOD

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ABSTRACT Moe P J and Seip M (Department of Paediatrics University of Tromsø Norway and Department of Paediatrics Rikshospitalet Oslo Norway) High-dose Methotrexate in acute lymphocytic leukemia in childhood Acta Paediatr Scand 67 265 1978.—Gonadal and other types of leukemic sanctuaries are probably the main causes of hematological relapse in the treatment of acute leukemia The introduction of high-dose Methotrexate (HDM) in a consolidation phase is based on theoretical considerations and the use of HDM in malignant tumors Three courses of Methotrexate 500 mg/sq m at 3-weekly intervals has been used as part of a consolidation therapy in Norway during the last two years to 59 children with ALL and one with AML One child died following HDM Post mortem examination showed that she was not in complete remission at the time Among 154 courses of HDM [redacted] the [redacted] patients were eight severe reactions including six cases of allergic toxi skin reactions Two patients developed a Stevens-Johnson s like syndrome Stomatitis was common in those with toxic reactions The risk of HDM [redacted] patients who are not [redacted] complete remission is stressed and the use of rescue therapy with two doses of Leukovorin instead of one is recommended Forty of forty two children in 1st complete remission have been in sustained primary remission for 4 to 28 months T[redacted] of these 40 children died after about a year from infections Only two patients so far have relapsed

KEY WORDS Childhood leukemia methotrexate leukovorin Stevens Johnson s syndrome

Central nervous system (CNS) prophylaxis has reduced the number of cases of CNS leukemia to 5–10 per cent in acute lymphocytic leukemia (ALL) in childhood (5) Conventional cytostatic therapy including CNS prophylaxis does not however prevent relapse in other sanctuaries

High dose Methotrexate (HDM) has the theoretical advantage of also hitting leukemic cells in neoplastic sanctuaries such as the gonads eyes spleen etc (2 7) Wang et al (7) conclude from pharmacological studies that Methotrexate given intrathecally is more effective in preventing CNS leukemia when given concomitantly with HDM intravenously

A clinical trial with HDM as consolidation therapy in acute lymphocytic leukemia was started in June 1975 Ten departments of pediatrics treating almost all cases of child hood leukemia in Norway are participating

The results of the pharmacological investigations concerning the use of Methotrexate will be published seperately (1) The purpose of this presentation will be to give a preliminary report of this study and [redacted] describe the side effects of this treatment regime

MATERIAL AND METHODS

Ten Norwegian pediatric departments participate in the HDM project A total of 59 children with ALL and one with AML received HDM as part of consolidation therapy Forty five were in their first remission 4 in their second remission and 11 had far advanced leukemia The

BOOK REVIEWS

J R Bierich K Rager & M B Ranke *Maldescensus testis* 198 pp illus Colloquium at Tubingen February 14 1976 Urban & Schwarzenberg Munchen Wien Baltimore 1977 DM 36 — ISBN 3 541-08031-0

The treatment of cryptorchidism has for many years been a matter of much controversy At what age should treatment be instituted should hormonal treatment be used in selected cases or is surgery always to be preferred what are the results with regard to future fertility are some important questions much discussed and to which definite answers have not yet been given A recent contribution to the discussion is a collection of papers presented at a meeting in Tübingen in February 1976 Thirty-one papers mostly by German authors deal with problems directly or in some cases indirectly connected with cryptorchidism The volume can be recommended to those who wish to orientate themselves about recent research within this field but those who are mainly concerned with practical therapeutic problems may be somewhat disappointed The main purpose of the colloquium was to establish an appropriate treatment plan for cryptorchidism but the reader will probably find himself still in doubt of the best way to treat these patients Only a small minority of the papers presented deal directly with the most important question namely fertility in adulthood after different modes of treatment

C G Bergstrand

S H Pierog & A Ferrara *Medical care of the sick newborn* 2nd ed 368 pp illus The C V Mosby Company Saint Louis 1976 US $14 50 ISBN 0-8016-3936-0

After five years Pierog & Ferrara present their second edition of Medical care of the sick newborn the first having been reviewed in Acta Paediatrica Scandinavica The changes made reflect the development in various neonatal subjects such as the changing ecology of the neonatal ward e g the increasing incidence of infections caused by group B beta haemolytic streptococcae seen in many centers and the growing number of infants to drug abusing mothers The chapters on metabolic problems respiratory disorders hematologic abnormalities and ventilatory therapy have been extended and brought up to date The book can be recommended as a compendium to the standard works in the neonatal field and it will be of great value for those who desire information about practical neonatal care

Per Henriksson

R I Mackay *Mental handicap in child health practice* 322 pp illus The Butterworth Group London 1976 £9 50 ISBN 0-407-00113 1

This book is written for doctors working with children The first chapter deals with definitions prevalence and trends The difficulties in using intelligence tests when defining mental handicap is illustrated It could have been more directly warned against the use of single tests which always give too high figures for mild mental handicap Performance at school gives a much more reliable basis for actions To stimulate the pediatricians interest the author should have pointed out from the beginning that mental handicap is by far the commonest form of handicap in children

The next two chapters deal with early identification prenatal and postnatal The author starts with an interesting (too short) description of the developing brain This is followed by descriptions and tables on inherited syndromes possible to detect prenatally and the methods used The parts on postnatal identification and clinical examination give short descriptions of a great many syndromes There are also many tables in which the examiner starts with an index symptom looks for associated symptoms and gets hints of first line studies in order to arrive at a correct diagnosis and aetiology This is a new and welcomed tool for workers in this field However such important causes as fetal infections and birth injury are not discussed When dealing with Prompt treatment behaviour problems constitute the more difficult part It should have been added here that the best way to handle such problems is to train these children in small groups adequately staffed

In the chapters Full assessment and Education from birth the author gives clear short descriptions and introductions with references The part on education refers to the works of Piaget but others are also quoted Guidance for parents is very clear and well written and is highly recommended

Finally the chapter dealing with Proper placement describes the organization of services in Great Britain Here a comparison with the facilities in some other countries well advanced in the education and care of children with mental handicap had been of value

This is a very useful book especially for those whose important task is to find the aetiology of the more severe forms of mental handicap

Mats Borjeson

Table 2 *Untoward reactions to HDM*

Pancytopenia + sepsis (?) + stomatitis	3
Skin reactions (Stevens Johnson s syndrome in ?) + stomatitis	6
Cytomegalovirus infection + stomatitis	1
Stomatitis in other cases	4
Total (out of 60 cases)	[illegible]

HDM well but she later died from sepsis following [illegible] course of COAP

Forty children in complete remission were followed from five months to 2½ years after the diagnosis with a median remission time of 12 months Only two of the 40 patients have relapsed so far One of them [illegible] in her second remission the other one died in relapse two years after the diagnosis was made Two children died in complete remission from infections about a year after their HDM treatment The remaining 36 cases have been in sustained primary remission for 4 to 24 months Furthermore two cases who received HDM prior to cessation of therapy are also in complete remission and off therapy making a total of 38 cases of HDM children alive in their first remission The child with AML has been in her first remission for 20 months So far no cases of CNS leukemia have been observed in the total 42 cases in 1st remission One of the four patients in 2nd remission had already experienced CNS leukemia the three remaining have not had CNS leukemia

The results of HDM in the four cases in second remission and the eleven cases with far advanced leukemia are more difficult to evaluate and are not too promising particularly not in far advanced cases

DISCUSSION

A more intensive prolonged induction or some new type of consolidation therapy is needed to substantially increase the cure rate in childhood leukemia For instance in our 8 years experience with the continuation of vincristine corticosteroids and L asparginase in the treatment of ALL lasting remissions occurred in only one third of the cases (4) More intensive induction treatments however carry a greater risk of serious reactions and these regimes are unlikely to prevent leukemia in the gonads or other sanctuaries

The introduction of HDM in a consolidation phase is a new approach in the treatment of this disease HDM has been an important part of intensive cytostatic therapy in osteogenic sarcoma and malignant lymphoma (6) In 1976 a group at Roswell Park Memorial Institute reported preliminary encouraging results from their use of HDM in ALL in children (2) Three courses of Methotrexate (500 mg/sq m/dose) were given at three weekly intervals in a 24 hour intravenous infusion followed by [illegible] single dose of Leukovorin They observed only minimal toxicity in 49 first line patients and severe reactions in one of 16 with far advanced leukemia (2 7) No case of AML was included in the report from Roswell Park Memorial Hospital We have so far found in this study only two relapses in 42 first line patients but the incidence of untoward reactions to HDM in our national study has been considerably higher than in the Roswell Park report Stomatitis was a frequent complication of HDM and skin reactions which seemed to be dose related occurred in 6 of our patients Stevens Johnson s syndrome a previously unreported complication of Methotrexate therapy occurred in two of those 6 children Pancytopenia accompanied by sepsis was found in 3 individuals Because of the seriousness of these reactions we increased the

Table 3 *Results [illegible] cases [illegible] 1st remission*

Died in incomplete remission	[illegible]
Persistent erythroid hyperplasia	1
Died after 1 year from infection	[illegible]
Relapse	2
Sustained primary remission	38
Total number [illegible] cases	45

Relapse rate of cases in complete primary remission 2/42=4 8% (followed for 4– 4 months)

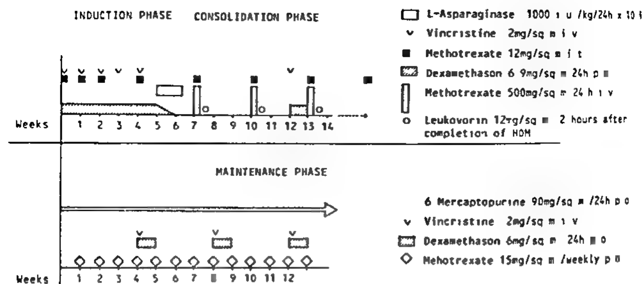


Fig 1 Treatment protocol for children with ALL

treatment protocol (Fig 1) for 37 of 45 newly diagnosed cases of ALL closely resembled that of Wang and his collegues (7) The induction phase consisted of vincristine and dexamethazone followed by L asparginase for 10 days In eight patients the protocol did not include L asparginase in seven of them 6-mercaptopurine was used in the induction phase in addition to vincristine and prednisone After remission had been achieved three intravenous courses of Methotrexate were administered at 3 weekly intervals One third of the dose was given in a half hour intravenous bolus and the remaining two thirds over a 24 hours infusion Twenty four hours following completion of the Methotrexate infusion a single dose of Leukovorin (citrovorum factor) was given using 12 mg/m² Since October 1976 two doses of Leukovorin have been used 12 and 24 hours after completion of Methotrexate infusion

Following the HDM courses maintenance therapy was started with daily oral 6-mercaptopurine and weekly oral Methotrexate and pulses of steroid/vincristine All the children received Methotrexate intrathecally (Fig 1) one combined with cranial irradiation Two children received only six doses of intrathecal Methotrexate Only one course of Methotrexate was given to the irradiated child due to the risk of combining irradiation with HDM

RESULTS

Up to the time of this report 154 courses of HDM had been administered to 59 patients with ALL and to one with AML (Table 1) The untoward reactions to this treatment are summarized in Table 3

A 7 year old female in partial remission died from pancytopenia and sepsis ten days following the first course of HDM The other 59 patients survived the courses of HD but serious reactions as well as stomatitis w observed in several cases (Table 2) Allerg toxic skin reactions occurred in 6 childr including Stevens Johnson s syndrome in t of them The dose of Methotrexate was duced in 4 patients due to previous adve reactions and further courses were disc tinued in 6 patients because of excessive to ity Two doses of Leukovorin have been u instead of one since October 1976

Liver function studies were not perform systematically No increase in transamin values were however observed in 13 patie following HDM

Three of the 45 1st line patients had o partial remission (Table 3) They included previously mentioned 7 year old female w died in sepsis a 10 month old infant with initial leukocyte count of 700 000/mm³ and a year old male child with a persistent mar erythroid hyperplasia The infant tolera

Table 1 *Survey of total material*

Primary remission	
(*a*) Complete	42
(*b*) Incomplete	3
Second remission	4
Far advanced leukemia	11
Total number of cases	60

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MVPP CHEMOTHERAPY COMBINED WITH RADIOTHERAPY IN THE TREATMENT OF HODGKIN S DISEASE IN CHILDREN

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K KACZOR and T DEPOWSKA

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ABSTRACT Armata J Stopyrowa J Depowski M Strzeszynski J Borkowski W Kaczor K and Depowska T (II Paediatric Clinic Institute of Paediatrics Copernicus Academy of Medicine Cracow Poland) MVPP chemotherapy combined with radiotherapy in treatment of Hodgkin s disease in children Acta Paediatr Scand 67 269 1978.—Thirty four children with Hodgkin s disease were treated during the years 1969–75 After radiotherapy 7–15 cycles of MVPP were given within 24–53 months In order to avoid chronic leukopenia leukocyte counts were made frequently during chemotherapy and the drug doses adjusted accordingly A complete remission was obtained in 32 of the 34 children Two patients died because of progressive disease Twelve of the 32 survivors have been followed for at least 5 years and a further 12 for at least 3 years Three children are still on chemotherapy whereas the remaining 29 being followed are in continued complete remission

KEY WORDS Childhood Hodgkin s disease MVPP chemotherapy radiotherapy

During the last decade the prognosis in Hodgkin s disease has improved dramatically both in adults (2 7 9 12 16 19 20 22 23) and in children (1 3 4 6 8 11 13 14 15 24 26 30 31) Today MOPP is usually reported as the chemotherapy of choice in Hodgkin s disease (7 12 13) but the use of vinblastine instead of vincristine (Oncovin®) in order to avoid neurotoxicity (MVPP) has also been described (9 19 20) We have used MVPP routinely since 1970 in children with Hodgkin s disease and the purpose of the present investigation was to analyse the results in 34 patients

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Abbreviations MOPP=Mustinehydrochloride Oncovin (vincristine) *Procarbazine Prednisone* MVPP=Mustine hydrochloride Vinblastine *Procarbazine Prednisone* Gy=Gray=100 rad

MATERIAL AND METHODS

From July 1969 through April 1975 34 consecutive children with Hodgkin s disease were treated at our Clinic The follow up was concluded on May 1st 1977 in order to obtain a minimum possible follow up of 2 years A follow up of 2 years is considered to be adequate for the evaluation of relapse free survival (6) The length of survival was determined in each patient from the time of initiating treatment to the time of death or the end of the study

The age of the patients ranged from 3 to 16 years with a median of 7 years There were 23 boys and 11 girls Seventeen boys and 7 girls (71%) came from rural areas

All patients had a biopsy proven diagnosis of Hodgkin s disease The biopsy specimens were classified according to the criteria of Lukes et al (18) At the time of analysis the patients were staged according to the Ann Arbor system (29)

The series of patients included 32 previously untreated children and 2 in first relapse (one after previous radiotherapy and one after previous radiotherapy and single drug chemotherapy) Medical history physical examination a complete blood count liver function tests and chest X ray were performed routinely whereas chest

rescue therapy in the protocol in late 1976 to two doses of leukovorin instead of the one recommended by Wang et al (7)

Encouraging preliminary results from the use of HDM have been obtained both at the Roswell Park Memorial Institute and in our national Norwegian study

A long term follow up of the patients in these studies will help to ascertain the efficiency of this new therapeutic approach Furthermore a large controlled study with and *without HDM as consolidation therapy* has been started in the United States under the auspices of CALGB Another 4–5 years however will be needed before definite conclusions can be drawn from that investigation Meanwhile pilot studies such as this one should be valuable in ascertaining the success or failure of this experimental protocol which *is designed to eradicate leukemia sanctuaries* and thereby significantly raise the cure rate in ALL in childhood

ACKNOWLEDGEMENTS

We wish to express our gratitude for information on patients participating in the study to the departments of pediatrics at the following hospitals Haukeland sykehus Regionsykehuset i Trondheim Ullevål sykehus Vestfold sentralsykehus Sentralsjukehuset i Rogaland Fylkessjukehuset i Ålesund Fylkessjukehuset i Kristiansund Innherred sykehus and Sentralsykehuset i Nordland

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Table 4 *Results of treatment in children with Hodgkin s disease*

Treatment started	Number of patients entered	Number of patients achieving a complete remission	Number of patients with primary failure	Number of patients with later relapse	Number of patients off therapy at the end of the follow up
1969	3	3	0	0	3
1970	2	2	0	1	1
1971	8	7	1	1	7
1972	3	3	0	0	3
1973	8	7	1	0	7
1974	8	8	0	1	7
1975	2	2	0	0	1
Total pats	34	32	2	3	29

Including 2 patients being treated for first relapse
Both decreased at the end of the follow up period

if there was a total disappearance of evidence of disease—physically radiologically and biochemically

After completion of therapy all patients were seen regularly at approximately 2 month intervals and evaluated for any re appearance of disease

RESULTS AND SIDE EFFECTS

Thirty two of the 34 patients (94%) had a complete remission and survived to the end of the follow up Two children did not respond and died with uncontrolled disease 2 years after the first treatment Twenty four patients had complete remission 3 months after the beginning of treatment and 8 patients after 4–8 months

Chemotherapy was concluded in 29 children Twenty eight of these were in primary remission and one was in second remission after intensified MVPP chemotherapy These 29 patients have been off chemotherapy for 2 to 57 months

Two children are still on DOPA therapy (17) for relapse One boy (stage IV) is still receiving MVPP therapy (Table 4)

The follow up periods for patients who had a complete initial remission appear in Table 5

The most common side effect of MVPP therapy was leukopenia (Table 6) but it was usually short lasting Some children had infections in association with leukopenia One child with prolonged leukopenia developed osteomyelitis which resulted in crippling In another child a boy of 5 years who had had a staging laparotomy with splenectomy purulent meningitis developed without preceding leukopenia The patient recovered after treatment with antibiotics

Vomiting was usually observed on the day of administration of nitrogen mustard No cases of thrombocytopenia neurological complications or alopecia were encountered in the present series Haematuria was seen in 2 chil

Table 5 *Follow up for 34 children with Hodgkin s disease*

Follow up	Number of patients
Complete remission	
More than 5 years	12
3–5 years	12
–3 years	8
No complete remission	
1 years	2 (both decreased)

Table 6 *Number of patients with pronounced drug induced leukopenia*

WBC/mm³	Number of patients
1 000–2 000	15
<1 000	12
Total	27

Table 1 *Distribution of 34 patients according to histological type and clinical stage*

Stage	Lymphocyte predominance (5 pats)	Mixed cellularity (22)	Lymphocyte depletion (5)	Nodular sclerosing type (0)	Not classified (2)
I (7 pats)	2	5	0	0	0
II (14)	2	10	0	0	2
III (10)	1	5	4	0	0
IV (3)	0	2[b]	1	0	0

One patient laparotomized [b] Two patients laparotomized [c] Three patients laparotomized

tomography was only done if mediastinal involvement was *present or suspected*

Seven children were subjected to a staging laparotomy with splenectomy In 3 of them the operation revealed previously undiagnosed involvement Laparotomized pa tients received prophylactic penicillin for a period of 2–3 years

The distribution of the patients by clinical stage and histological type is set out in Table 1

*All patients were treated with a combination of radio-*therapy and MVPP chemotherapy In 85% of the children radiotherapy was given before chemotherapy

Sometimes in advanced Hodgkin s disease the MVPP chemotherapy was the primary treatment (Table 2) MVPP chemotherapy was started during radiotherapy in 6 cases These include 2 children with a first relapse and 4 others in stage III In one case (stage IV) treatment was begun with radiotherapy and another child with stage IV had palliative radiotherapy later in the course of disease

The radiotherapy was given with conventional (210 kV) X rays *For mediastinal and abdominal involvement* a tumour dose of 30 Gy in 5–6 weeks was given and for superficial lymph node regions the peak absorbed dose (surface abs dose) was 39 Gy given in 2 5–3 5 weeks Extended field radiotherapy was never used

MVPP chemotherapy was usually started one month after the conclusion of radiotherapy In children who started chemotherapy in 1969 or early 1970 only vin blastine procarbazine and prednisone were given but later maintenance therapy was given with complete MVPP cycles Patients who started chemotherapy after late 1970 received full *MVPP chemotherapy*

In the present series the original MVPP schedule (9 III 20) was modified as follows

Table 2 *Number of patients with un irradiated lesions at commencement of MVPP chemo therapy*

Stage	Number of patients
I	0/7
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III	2/10
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1 Vinblastine was injected on the 1st and 8th days i *single dose of 0 2 mg/kg bodyweight*

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4 *The first three MVPP cycles* were given with week interval between beginning of the cycles The nu ber of further cycles and interval between them depen on the clinical stage The average number of MVPP cy was 7 for patients in stages I and II and 11 in stages and IV

5 In some patients additional vinblastine was gi monthly in intervals between the MVPP cycles

During the years 1969–72 7–15 (mean 10) MVPP cy were given for 24–53 (mean 42) months Three patie received only a few cycles since the parents only ga *consent for short combination chemotherapy* These c dren however later received Vinblastine monthly

During 1973–74 the same number of MVPP cycles w still given but the overall duration of chemotherapy shortened to a mean of 29 months

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Table 3 *Dose reduction schedule of MV according to WBC counts before and duri the cycles*

WBC/mm³	Dosage of drugs
≥4 000	100% of all
3 999–3 000	100% Vinblastine 50% Nitrogen mustard and Procarbazine
2 999–2 000	100% Vinblastine 25% Nitrogen mustard and Procarbazine
1 999–1 000	50% Vinblastine None Nitrogen mustard or Procarbazine
<1 000	No drugs

Table 4 *Results of treatment in children with Hodgkin s disease*

Treatment started	Number of patients entered	Number of patients achieving a complete remission	Number of patients with primary failure	Number of patients with later relapse	Number of patients off therapy at the end of the follow up
1969	3	3	0	0	3
1970	?	2	0	1	1
1971	8	7	1	1	7
197?	3	3	0	0	3
1973	8	7	1	0	7
1974	8	8	0	1	7
197?	2	2	0	0	1
Total pats	34	3?	2*	3	?9

Including 2 patients being treated for first relapse
Both decreased at the end of the follow up period

if there was a total disappearance of evidence of disease—physically radiologically and biochemically

After completion of therapy all patients were seen regularly at approximately 2 month intervals and evaluated for any re appearance of disease

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dren Azospermia was found in 1 patient but not examined for in the other boys because of their low age

Radiation pneumonitis was seen in 3 children Two other children had an acute laryngeal radiation reaction after irradiation of the cervical region and in one of them it was necessary to perform a tracheostomy

DISCUSSION

Most of the children came from rural areas namely 64% of the girls and 74% of the boys In children Hodgkin s disease has been reported to occur more frequently in urban than in rural areas but this may not be true for boys (10)

The distribution of histological types in Hodgkin s disease in children has been reported to vary from one geographic area to another Of the children in the present series, 65% had mixed cellularity which has been reported to be the most common type in Europe in the paediatric age group (5 8, 11 13 15 27) On the other hand the nodular sclerosing type has been reported to be the most frequent in the US and Canada (6 13 14 21 24 25 26) Usually the prognosis is worse for mixed cellularity than for the nodular sclerosing type (28), but this has not been confirmed by studies of paediatric series (6 21)

The most frequently reported combination chemotherapy in Hodgkin s disease in children is the Vincristine combination MOPP (1 4 6 13 14, 15 30) and remission rates of 100% after MOPP have been reported (31) However it has also been claimed that MOPP is not so effective in children as in adults (30)

Results of other types of combination chemotherapy have also been given (24 26)

In the present series a combination of radiotherapy and MVPP resulted in a complete remission rate of 94% for all stages Three children have had a successful reinduction with MVPP after their first relapse Twenty nine children remain in remission without therapy after a follow up of 2–57 months Twelve have been followed for 3–5 years and 12 for at least 5 years

Drug dosage was reduced according to WBC counts prior to and during each cycle of MVPP Frequent WBC counts during a cycle allowed for a better evaluation of bone marrow function than only WBC counts prior to th MVPP cycle and facilitated monitoring of chemotherapy in order to avoid chronic bone marrow depression Usually the amount of procarbazine was reduced and this probably explains why thrombocytopenia which has been reported to be common (4 7 16 19 20) was not encountered in the present series

In the present series serious complications were rare and only seen in one patient in association with prolonged neutropenia There were no therapy related deaths

The present series indicates that radiotherapy combined with MVPP chemotherapy appears to be effective in Hodgkin s disease in children

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ANTIBODY COATED BACTERIA IN THE URINE OF INFANTS AND CHILDREN WITH THEIR FIRST TWO URINARY TRACT INFECTIONS

J PYLKKANEN

From the Children s Hospital University of Helsinki Finland

ABSTRACT Pylkkanen J (The Children s Hospital University of Helsinki Finland) Antibody-coated bacteria in the urine of infants and children with their first two urinary tract infections Acta Paediatr Scand 67 275 1978.—The presence of antibody-coated bacteria in urine has been shown to be an indicator for renal bacteriuria in adults with chronic UTI To evaluate this method (ABCU test) in pediatric patients 128 infants and children with their first UTI were investigated Twenty nine patients of the 78 who had a first clinically defined upper UTI had antibody-coated bacteria in urine The test was seldom (2/20) positive in the infants under six months In the older patients (27/58 ABCU positive) the frequency of positive tests increased with the duration of symptoms When the symptoms had lasted for over one week 11 out of the 13 children with their first upper UTI showed antibody-coated bacteria in urine Four of the 36 first lower UTIs and 5 out of the 14 asymptomatic cases were ABCU positive The patients were followed up for an average of 9 months Those who were classified as having first upper UTI had in most cases a positive ABCU test in recurrences independently of the clinical picture The recurrences after the first lower UTIs showed antibody-coated bacteria in urine only when the recurrence was classified as upper UTI on the grounds of the clinical criteria used

KEY WORDS Antibody-coated bacteria urinary tract infection

Most cases of atrophic pyelonephritis have their origin in the first decade of life (4) Therefore the anatomical level of urinary tract infections (UTIs) in infancy and childhood is of special importance In practice the level diagnosis of UTIs is difficult due to lack of simple and reliable methods It has been maintained that symptomatic upper and lower UTIs can be distinguished by combining the information of the clinical picture and routine laboratory tests with serum antibody titrations against the infecting organism (6) With certain symptomatic patients however these parameters give contradictory results In asymptomatic bacteriuria (ABU) useful methods have not been available for the determination of the level of UTI (9)

Thomas et al introduced the test for antibody-coated bacteria in urine (ABCU test) for the detection of renal bacteriuria (11) My article reports on the occurrence of antibody coated bacteria in the first two UTIs of 128 infants and children The aims of this study are 1) to compare the clinical level diagnosis with the result of the ABCU test in first UTIs 2) to investigate the influence of the age of the patient and the duration of the symptoms on the presence of antibody coated bacteria in the urine of the first UTIs 3) to compare the clinical level diagnosis and the result of the ABCU test in first UTIs with the corresponding information in second UTIs

MATERIAL AND METHODS

One hundred and fourteen symptomatic and 14 asymptomatic patients with their first detected UTI were in-

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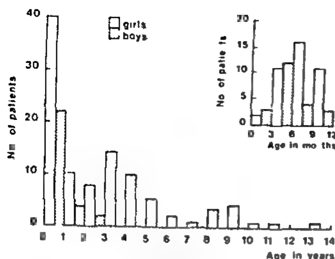


Fig 1 Age and sex distributions of the patients at the time of the first UTI *The graph on top right shows the* subdivision of the infants in four age groups

cluded in the study The sex and age distributions appear in Fig 1

The diagnosis of the first UTI was made by means of suprapubic bladder aspiration (56 patients) or through showing significant bacterial growth (≥10⁵ org /ml) of the same species and more than 20 Leukocytes/mm³ in repeated midstream urine samples or bag specimens (72 patients) Prior to the conventional urine samples the *outer genitalia were gently washed with sterile water*

The clinical level diagnosis of symptomatic UTIs was based on the modified criteria of Jodal et al (6) The patient was classified as having acute upper UTI when *3 of the following four criteria were fulfilled*

1 High fever (Tax≥39 0 or Trect≥39 5)

2 Increased erythrocyte sedimentation rate (≥35 mm/h with a macromethod)

3 Abnormally high level of C reactive protein (≥25 μg/ml)

4 Decreased renal concentrating capacity in relation to age

Correspondingly the patient was classified in the group of lower UTI if 2 or less of the criteria were fulfilled All patients with decreased renal concentrating capacity and high level of C reactive protein fulfilled at least 3 criteria With asymptomatic patients the clinical level diagnosis was not made

Quantitative determination of C reactive protein was performed through single radial immunodiffusion using specific antisera (Hyland Los Angeles) The maximal renal *concentrating capacity* was determined 5 to 10 days after the onset of the therapy mostly by using 1-de amino-8 D-arginine vasopressin (DDAVP) an analogue of the antidiuretic hormone (2) In 18 infants renal concentrating capacity was estimated after 16 hours fluid deprivation under close hospital surveillance The osmolality in 3 consecutive urine samples was measured by a freezing point osmometer The highest value achieved was compared with the normal values given by Winberg (14)

The test for the detection of antibody-coated bacteria in urine (ABCU test) was performed by the method of *Thomas et al (11)* Fluorescein-conjugated goat antiserum to human globulin (Hyland Los Angeles) was used In some patients the samples were also investigated by fluorescein-conjugated monospecific antisera (goat) to *human IgG IgM and IgA (Hyland Los Angeles)* The ABCU tests were read without prior knowledge of the clinical level diagnosis of each patient The fluorescence was considered positive if a minimum of two brightly *fluorescent bacteria could be found in the specimen* Controls were prepared from 53 specimens each time a new lot of antiserum was taken into use The antisera were free of antibody against each subcultured bacterial isolate and conjugated antihuman globulin blocked the fluorescence in all investigated specimens

The patients were treated with sulfafurazole (?00 mg/kg/day) or nitrofurantoin (3 mg/kg/day) After the cessation of the therapy they were regularly controlled for significant bacteriuria by Uricult® dip slides (Orion Pharmaceutical Co Helsinki)

RESULTS

I *Antibody coated bacteria in first UTIs*

The clinical level diagnosis was compared with the result of the ABCU test in first UTIs

A *Clinically defined upper UTIs*

Twenty nine patients out of the 78 who had a first clinically upper UTI had antibody coated bacteria in urine (Table 1) Among infants under six months only 2 of the 20 had a posi

Table 1 *First UTIs Clinical level diagnosis and result of the ABCU test*

Clinical level diagnosis of the first UTI	Age at the time of the first UTI	Result of the ABCU test positive/no of patients
Upper UTI	< 6 0 months	?/20
	6 0–11 9 months	9/23
	≥12 0 months	18/35
	Total	29/78
Lower UTI	< 6 0 months	0/ 8
	6 0–11 9 months	0/ 9
	≥12 0 months	4/19
	Total	4/36
ABU	< 6 0 months	0/ 0
	6 0–11 9 months	0/ 2
	≥12 0 months	5/12
	Total	5/14

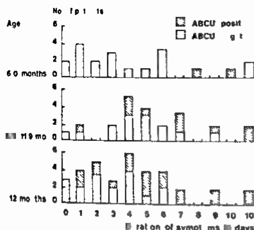


Fig 2 First clinically upper UTIs Duration of symptoms and result of the ABCU test

tive ABCU test Twenty seven of the 58 older patients were ABCU positive

The duration of symptoms in the ABCU positive and ABCU negative cases is presented in Fig 2 In the lowest age group both the 2 ABCU positive patients had symptoms for over one week Among the older children the test was sometimes positive during the first week after the onset of symptoms but when the clinical disease had lasted longer 11 patients out of the 13 had antibody coated bacteria in urine

B *Clinically defined lower UTIs*

Four children with their first clinically lower UTI showed a positive ABCU test Each one had symptoms for at least 5 days before the preparation of the specimen

C *ABUs*

Five of the 14 patients had antibody coated bacteria in urine All of these were girls

D *Class of antibody*

In 16 symptomatic patients the class of antibody coating the bacteria in urine was documented (Table 2) IgG was present in all but one specimen IgA in one half of the samples IgM was found in one patient only

II *Antibody coated bacteria in second UTIs*

The patients were observed for an average of 9 months They were divided into 6 subgroups according to their clinical level diagnosis and the result of the ABCU test in the first UTI The corresponding information concerning the second UTIs is presented in Table 3

A *Patients whose first UTI was classified as clinically upper UTI*

Twenty four (31%) patients who had a first clinically upper UTI had a recurrence during the follow up Twenty one recurrences were investigated for the presence of antibody coated bacteria in urine *Patients who had antibody coated bacteria in urine during the first UTI* had a ABCU positive recurrence in 6 cases out of 7 (Table 3) Three of these were asymptomatic Thus if the ABCU test was positive in first UTI it tended to be positive again during the second infection independently of the clinical picture of the recurrence

Among patients with a first clinically upper UTI but a negative ABCU test 14 recurrences were investigated Eight of these showed antibody coated bacteria in urine Half of the asymptomatic recurrences were again ABCU positive

B *Patients whose first UTI was classified as clinically lower UTI*

Seven (19%) patients with their first clinically lower UTI had a recurrence during the follow up A positive ABCU test was observed in

Table 2 *Class of antibody coating the bacteria in the urine of sixteen first symptomatic UTIs*

Immunoglobulins in positive ABCU tests	No of patients
IgG	7
IgG+IgA	7
IgG+IgA+IgM	1
IgA	1
Total	16

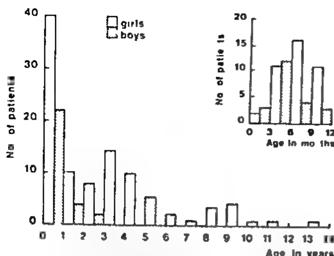


Fig 1 Age and sex distributions of the patients at the time of the first UTI The graph on top right shows the subdivision of the infants in four age groups

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The diagnosis of the first UTI was made by means of suprapubic bladder aspiration (56 patients) or through showing significant bacterial growth (≥10^5 org /ml) of the same species and more than 20 Leukocytes/mm^3 in repeated midstream urine samples or bag specimens (72 patients) Prior to the conventional urine samples the outer genitalia were gently washed with sterile water

The clinical level diagnosis of symptomatic UTIs was based on the modified criteria of Jodal et al (6) The patient was classified as having acute upper UTI when 3 of the following four criteria were fulfilled

1 High fever (Tax≥39 0 or Trect≥39 5)

2 Increased erythrocyte sedimentation rate (≥35 mm/h with a macromethod)

3 Abnormally high level of C reactive protein (≥25 μg/ml)

4 Decreased renal concentrating capacity in relation to age

Correspondingly the patient was classified in the group of lower UTI if 2 or less of the criteria were fulfilled All patients with decreased renal concentrating capacity and high level of C reactive protein fulfilled at least 3 criteria With asymptomatic patients the clinical level diagnosis was not made

Quantitative determination of C reactive protein was performed through single radial immunodiffusion using specific antisera (Hyland Los Angeles) The maximal renal concentrating capacity was determined 5 to 10 days after the onset of the therapy mostly by using 1 de amino 8 D-arginine vasopressin (DDAVP) an analogue of the antidiuretic hormone (2) In 18 infants renal concentrating capacity was estimated after 16 hours fluid deprivation under close hospital surveillance The osmolality in 3 consecutive urine samples was measured by a freezing point osmometer The highest value achieved was compared with the normal values given by Winberg (14)

The test for the detection of antibody coated bacteria in urine (ABCU test) was performed by the method of Thomas et al (11) Fluorescein conjugated goat antiserum to human globulin (Hyland Los Angeles) was used In some patients the samples were also investigated by fluorescein conjugated monospecific antisera (goat) to human IgG IgM and IgA (Hyland Los Angeles) The ABCU tests were read without prior knowledge of the clinical level diagnosis of each patient The fluorescence was considered positive if a minimum of two brightly fluorescent bacteria could be found in the specimen Controls were prepared from 53 specimens each time a new lot of antiserum was taken into use The antisera were free of antibody against each subcultured bacterial isolate and conjugated antihuman globulin blocked the fluorescence in all investigated specimens

The patients were treated with sulfafurazole ([illegible]00 mg/kg/day) or nitrofurantoin (3 mg/kg/day) After the cessation of the therapy they were regularly controlled for significant bacteriuria by Uricult® dip slides (Orion Pharmaceutical Co Helsinki)

RESULTS

I Antibody coated bacteria in first UTIs

The clinical level diagnosis was compared with the result of the ABCU test in first UTIs

A Clinically defined upper UTIs

Twenty nine patients out of the 78 who had a first clinically upper UTI had antibody coated bacteria in urine (Table 1) Among infants under six months only 2 of the 20 had a posi

Table 1 *First UTIs Clinical level diagnosis and result of the ABCU test*

Clinical level diagnosis of the first UTI	Age at the time of the first UTI	Result of the ABCU test positive/no of patients
Upper UTI	< 6 0 months	2/20
	6 0–11 9 months	9/23
	≥12 0 months	18/35
	Total	29/78
Lower UTI	< 6 0 months	0/ 8
	6 0–11 9 months	0/ 9
	≥12 0 months	4/19
	Total	4/36
ABU	< 6 0 months	0/ 0
	6 0–11 9 months	0/ 2
	≥12 0 months	5/12
	Total	5/14

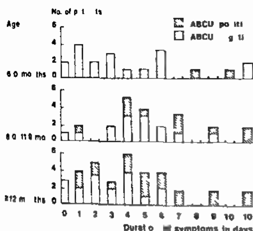


Fig 2 First clinically upper UTIs Duration of symptoms and result of the ABCU test

tive ABCU test Twenty seven of the 58 older patients were ABCU positive

The duration of symptoms in the ABCU positive and ABCU negative cases is presented in Fig 2 In the lowest age group both the 2 ABCU positive patients had symptoms for over one week Among the older children the test was sometimes positive during the first week after the onset of symptoms but when the clinical disease had lasted longer 11 patients out of the 13 had antibody coated bacteria in urine

B *Clinically defined lower UTIs*

Four children with their first clinically lower UTI showed a positive ABCU test Each one had symptoms for at least 5 days before the preparation of the specimen

C *ABUs*

Five of the 14 patients had antibody coated bacteria in urine All of these were girls

D *Class of antibody*

In 16 symptomatic patients the class of antibody coating the bacteria in urine was documented (Table 2) IgG was present in all but one specimen IgA in one half of the samples IgM was found in one patient only

II *Antibody coated bacteria in second UTIs*

The patients were observed for an average of 9 months They were divided into 6 subgroups according to their clinical level diagnosis and the result of the ABCU test in the first UTI The corresponding information concerning the second UTIs is presented in Table 3

A *Patients whose first UTI was classified as clinically upper UTI*

Twenty four (31%) patients who had a first clinically upper UTI had a recurrence during the follow up Twenty one recurrences were investigated for the presence of antibody coated bacteria in urine *Patients who had antibody coated bacteria in urine during the first UTI* had a ABCU positive recurrence in 6 cases out of 7 (Table 3) Three of these were asymptomatic Thus if the ABCU test was positive in first UTI it tended to be positive again during the second infection independently of the clinical picture of the recurrence

Among patients with a first clinically upper UTI but a negative ABCU test 14 recurrences were investigated Eight of these showed antibody coated bacteria in urine Half of the asymptomatic recurrences were again ABCU positive

B *Patients whose first UTI was classified as clinically lower UTI*

Seven (19%) patients with their first clinically lower UTI had a recurrence during the follow up A positive ABCU test was observed in

Table 2 *Class of antibody coating the bacteria in the urine of sixteen first symptomatic UTIs*

Immunoglobulins in positive ABCU tests	No of patients
IgG	7
IgG+IgA	7
IgG+IgA+IgM	1
IgA	1
Total	16

Table 3 *Second UTIs Clinical level diagnosis and result of the ABCU test*

The patients are divided into six subgroups according to their clinical level diagnosis and the result of the ABCU test in the first UTI

First UTIs		Second UTIs	
Clinical level diagnosis	Result of the ABCU test	Clinical level diag nosis	Result of the ABCU test positive/no of patients
Upper UTI	Positive	Upper UTI	2/ 2
		Lower UTI	1/ 2
		ABU	3/ 3
		Total	6/ 7
	Negative	Upper UTI	4/ 4
		Lower UTI	1/ 4
		ABU	3/ 6
		Total	8/14
Lower UTI	Positive	Upper UTI	0/ 0
		Lower UTI	0/ 0
		ABU	0/ 0
		Total	0/ 0
	Negative	Upper UTI	1/ 1
		Lower UTI	0/ 4
		ABU	0/ 2
		Total	1/ 7
ABU	Positive	Upper UTI	0/ 0
		Lower UTI	0/ 0
		ABU	2/ 2
		Total	2/ 2
	Negative	Upper UTI	1/ 1
		Lower UTI	0/ 0
		ABU	0/ 2
		Total	1/ 3

one case only (Table 3) This patient had high fever for two days but other level diagnostic parameters were not documented

C *Patients whose first UTI was classified as ABU*

Five recurrences occurred among these patients The results of the ABCU test did not differ from those of the first UTI but in one case (Table 3) A fulminant clinically upper UTI turned the test positive in a girl whose first ABU had been treated 7 months earlier at the age of 9 months

DISCUSSION

Thomas et al (11) demonstrated the presence of antibody coated bacteria in the urine of patients with UTI A clear correlation between the results of this method and the bladder washout technique was observed in adults with chronic UTI (7 8) Antibody coated bacteria have also been found in symptomatic UTIs of children (3) and pregnant women (13)

The presence of antibody coated bacteria does not depend on the total level of immunoglobulins in the urine (12) The production of the coating antibodies correlates with the local synthesis of antibody against the O antigen of the infecting organism (10)

Twenty nine of the 78 patients with their first clinically upper UTI had antibody coated bacteria in urine On the other hand, the patients with their first lower UTI only occasionally showed a positive test The frequent negative tests in clinically upper UTIs can be explained partly on the basis of the slowness of the primary immunological response The test was in general negative during the first days of the clinical disease but when the symptoms had lasted for one week 13 out of 17 patients had antibody coated bacteria in urine In experimental pyelonephritis antibody coated bacteria were not found until the 11th day of the infection (10)

The age of the patients also clearly influenced the result of the ABCU test Among the infants of under 6 months the test was seldom positive (2/20) in contrast to the first clinically upper UTIs of older patients (27/58) The result suggests that the primary immunological response producing antibodies coating the bacteria is slower and weaker during the first 6 months than later in life

Five of the 14 patients with their first ABU had antibody coated bacteria in urine The percentage of renal affection in ABU has generally been estimated lower (7) The number of my patients is however insufficient for any conclusions

If antibody-coated bacteria were found during the first UTI the ABCU test was generally positive also in recurrences irrespectively of the clinical level diagnoses In the patients with their first clinically upper UTI but a negative ABCU test 8 out of 14 recurrences were ABCU positive Three of the 6 symptomatic recurrences of these patients showed antibody-coated bacteria in urine On the other hand the recurrences after the first clinically lower UTIs were again ABCU negative and no discrepancy to the clinical level diagnosis of recurrences appeared

The recurrences after the first clinically upper UTIs most often show a positive ABCU test suggesting renal involvement independently of the clinical picture of recurrences Firm conclusions on the anatomical level of asymptomatic recurrences cannot be made according to the routine laboratory methods The patients with a positive ABCU test may form a risk group for renal damage This can only be evaluated after a follow up of several years of these patients

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		Lower UTI	1/ 2
		ABU	3/ 3
		Total	6/ 7
	Negative	Upper UTI	4/ 4
		Lower UTI	1/ 4
		ABU	3/ 6
		Total	8/14
Lower UTI	Positive	Upper UTI	0/ 0
		Lower UTI	0/ 0
		ABU	0/ 0
		Total	0/ 0
	Negative	Upper UTI	1/ 1
		Lower UTI	0/ 4
		ABU	0/ 2
		Total	1/ 7
ABU	Positive	Upper UTI	0/ 0
		Lower UTI	0/ 0
		ABU	2/ 2
		Total	2/ 2
	Negative	Upper UTI	1/ 1
		Lower UTI	0/ 0
		ABU	0/ 2
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one case only (Table 3) This patient had high fever for two days, but other level diagnostic parameters were not documented

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		ABU	3/ 3
		Total	6/ 7
	Negative	Upper UTI	4/ 4
		Lower UTI	1/ 4
		ABU	3/ 6
		Total	8/14
Lower UTI	Positive	Upper UTI	0/ 0
		Lower UTI	0/ 0
		ABU	0/ 0
		Total	0/ 0
	Negative	Upper UTI	1/ 1
		Lower UTI	0/ 4
		ABU	0/ 2
		Total	1/ 7
ABU	Positive	Upper UTI	0/ 0
		Lower UTI	0/ 0
		ABU	2/ 2
		Total	2/ 2
	Negative	Upper UTI	1/ 1
		Lower UTI	0/ 0
		ABU	0/ 2
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BLOOD VOLUME OF CHILDREN WITH LEUKEMIA

O LINDERKAMP B LAU K P RIEGEL and K BETKE

From the Department of Paediatrics University Hospital Munich Federal Republic of Germany

ABSTRACT Linderkamp O Lau B Riegel K P and Betke K (Department of Paediatrics University Hospital Munich Federal Republic of Germany) Blood volume of children with leukemia Acta Paediatr Scand 67 281 1978.—Blood volume was measured using ¹²⁵iodinated human serum albumin in 27 children with acute lymphoblastic leukemia and in 7 children with various types of leukemia Total blood volume was normal in patients without marked enlargement of spleen and liver and increased progressively as spleen and liver size increased The hypervolemia was entirely due to expansion of plasma volume In the children with marked hepatosplenomegaly only hematocrit (but not red cell mass) was below the normal range in most cases Both hematocrit and red cell mass were subnormal in the majority of patients without considerably enlarged spleen and liver Therefore anemia in children with marked hepatosplenomegaly may be partly caused by hemodilution of red blood cells in expanded plasma volume

KEY WORDS Blood volume leukemia splenomegaly hepatomegaly childhood

Anemia and hepatosplenomegaly are common findings in children suffering from leukemia Blood volume studies in adult patients with splenomegaly due to myeloproliferative and lymphoproliferative disorders have revealed that blood volume and plasma volume increase in relation to spleen size whereas red cell mass is less affected resulting in hemodilutional anemia (3 4 8 11 21) We are not aware of any report on blood volume data of children with leukemia Knowledge of blood volume in leukemic children is important since these patients frequently require blood transfusions The quantity of red blood cells that must be transfused in order to increase hematocrit to a desired value depends on the blood volume

The purpose of the present study was to evaluate whether in leukemic children a relation between spleen size and blood volume similar to that in adult patients could be found and whether blood volume in these patients could be predicted from spleen and liver size

PATIENTS AND METHODS

Thirty four leukemic children aged 9 months to 16 years were studied 27 had acute lymphoblastic leukemia 3 acute granulocytic leukemia 3 chronic granulocytic leukemia and 1 acute monocytic leukemia None of the patients received transfusions within a week prior to the test Hepatic size was measured vertically on the mid clavicular line from the costal margin to the lower edge of the liver Spleen size was also measured vertically from the costal margin on the left anterior axillary line to the spleen tip

The microhematocrit level was determined in duplicate and corrected for 2% of trapped plasma (16) Plasma volume was measured by injecting 0 075 μCi/kg ¹²⁵iodinated human serum albumin (Squibb New Brunswick N J USA) resulting in a radiation dose of 0 8 mrad (10) Three to six blood samples were taken within one hour The radioactivity of 0 5 ml of each plasma sample was measured in a well type scintillation counter (Frieseke & Hoepner Erlangen Bruck FRG) The counts were retropolated to zero time (16) Blood volume and red cell mass were calculated from plasma volume and hematocrit using a body/venous hematocrit ratio of 0 91 (5 6 15) The thyroid gland was blocked by administra

19 78 873

Table [illegible] *Correlations between excess blood volume (E BV) and spleen (S) and liver (L) [illegible] e*[a]

Regression equation	*s*	*r*
E BV[b] (ml)=57+68 3 S	303	0 69
E BV[b] (ml)=−71+63 3 (S+L)	?78	0 75
E BV (%)=2 7+3 66 S	11 3	0 81
E BV (%)=−3 7+3 [illegible] (S+L)	10 6	0 83
E BV (%)=? 7+431 S/H[d]	10 7	0 83
E BV (%)=−3 0+368 (S+L)/H[d]	10 1	0 85

Spleen and liver size measured in cm
[b] Difference between the measured and the predicted blood volumes
Difference between the measured and the predicted blood volumes expressed as a percentage of the predicted blood volume
H=height in cm

hematocrit was below normal range in the majority of patients independent of the degree of splenic and hepatic enlargement Red cell mass was decreased in only 3 of 12 patients with marked hepatosplenomegaly whereas it was below the normal range in 10 of 22 children without considerably enlarged spleen and liver Accordingly the mean red cell mass of the patients with marked hepatosplenomegaly (26 7±8 7 ml/kg) was significantly higher ($p<$ 0 025) than the mean value of the children without marked hepatosplenomegaly (20 8± 6 0 ml/kg) although the mean hematocrit values were similar in both groups (0 282± 0 078 and 0 295±0 083 respectively)

DISCUSSION

The calculation of total blood volume and red cell mass using measurement of plasma volume and hematocrit was based on the assumption of a constant body/venous hematocrit ratio The ratio of 0 91 used in our study has been found to be constant over a wide hematocrit range in newborn infants and adults (5 6) In adult patients the body/venous hematocrit ratio has been shown to increase in relation to spleen size (9 11 18) A similar relationship between the body/venous hematocrit ratio and spleen size has been found by our working group in a study on 7 children with acute lymphoblastic leukemia (15) The wide range of the data reported in the literature does not allow prediction of the true individual values We have therefore used the same ratio of 0 91 for all patients Provided that the body/venous hematocrit ratio in our splenomegalic patients was higher than 0 91 the blood volume in these patients was underestimated This implies an even greater hypervolemia in the patients with marked enlargement of the spleen as estimated

Increased blood volumes and plasma volumes due to splenomegaly have been found by other authors in adult patients with lymphoproliferative and myeloproliferative disorders (1–4 7 [illegible] 12 13 19–22) In these studies liver size has not been considered Our results indicate that blood volume in leukemic children is substantially influenced by spleen and liver size

Anemia is a predominant symptom in childhood leukemia It may be caused by splenic pooling of red blood cells shortened red cell survival time or depressed erythropoiesis (7 23) In hepatomegaly blood pooling in the liver and in the portal vein area may occur (14) According to our findings hemodilution due to increased plasma volume can be con

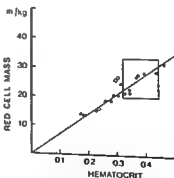


Fig 2 Relationship between red cell mass and hematocrit in patients with spleen plus liver sizes per height of more than 0 05 (O) and less than 0 05 cm/cm (●) Line represents relationship between red cell mass and hematocrit for a normal blood volume of 77 ml/kg (17) calculated as a product of normal blood volume hematocrit and the factor 0 91 The boxed area shows the normal range (±? S D)

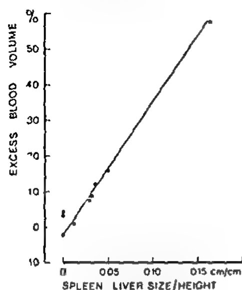


Fig 1 Relationship of blood volume expressed as a percentage of the predicted blood volume to spleen plus liver size related to height

tion of Lugol s solution before and for 5 days after injection of the radionuclide Normal blood volumes were derived from nomograms (17)

RESULTS

In 26 of the 34 patients the blood volume was greater than that predicted from nomograms for normal blood volumes In 15 children blood volume was found to be over 10% in excess of normal values (Fig 1) Table 1 summarizes the blood volume data in relation to the types of leukemia and to the stage of disease in the children with acute lymphoblastic leukemia Low red cell mass and hematocrit values were found in the children with acute lymphoblastic leukemia before treatment during induction therapy and in those with relapses Plasma volume and blood volume were considerably increased in the children with acute lymphoblastic leukemia during induction therapy and in the patients with various types of leukemia The majority of these children showed marked enlargement of the spleen and/or liver whereas only a few of the children with acute lymphoblastic leukemia in remission or with relapse had enlarged spleen or liver This indicates that hepatosplenomegaly was responsible for the hypervolemia

Correlations between excess blood volume (i e difference between measured and predicted blood volumes) and spleen and liver size are summarized in Table 2 The correlations were highly significant ($p<0\ 001$) The highest correlation coefficient was found when excess blood volume expressed as a percentage of the predicted blood volume was related to spleen plus liver size per cm of height ($r=0\ 85$) Fig 1 shows that blood volume increased linearly as spleen and liver size per cm of height increased

Fig 2 shows the relationship between red cell mass and hematocrit in the patients with marked hepatosplenomegaly and those with moderately or without enlarged spleen and liver In most cases the red cell mass of patients with marked hepatosplenomegaly was considerably higher than expected from their hematocrit Moreover Fig 2 illustrates that

Table 1 *Blood volume data in children with leukemia (x±S D)*

Disorder stage	n	Age (years)	Hematocrit	Plasma volume (ml/kg)	Red cell mass (ml/kg)	Blood volume (ml/kg)
ALL						
Before treatment	9	1–11	0 287±0 068	63 0±13 5	21 6± 4 5	[redacted] 6±13 0
Induction therapy	4	9/12–14	0 226±0 079	77 9±24 9	19 5± 7 4	97 4±26 3
Remission	9	3–10	0 349±0 060	54 3± 6 6	25 2± 4 2	79 5± 5 5
Relapse	5	5–13	0 231±0 060	61 4± 7 6	16 3± 4 0	77 7± 7 2
AGL CGL AML	7	2–16	0 300±0 089	71 7± 7 0	28 3±11 4	100 0±16 1
Normal	93	9/12–14	0 396±0 042	49 8± 6 7	27 0± 3 6	76 8± 8 6

ALL=acute lymphoblastic leukemia AGL=acute granulocytic leukemia CGL=chronic granulocytic leukemia AML=acute monocytic leukemia

Table 2 *Correlations between excess blood volume (E BV) and spleen (S) and liver (L) size*[a]

Regression equation	s	r
E BV[b] (ml)=57+68 3 [illegible]	303	0 69
E BV[b] (ml)=−71+63 3 (S+L)	278	0 75
E BV (%)=? 7+3 66 S	11 3	0 81
E BV (%)=−3 7+3 [illegible] (S+L)	10 6	0 83
E BV (%)=? 7+431 S/H[d]	10 7	0 83
E BV (%)=−3 0+368 (S+L)/H[d]	10 1	0 85

Spleen and liver size measured in cm

Difference between the measured and the predicted blood volumes

Difference between the measured and the predicted blood volumes expressed as a percentage of the predicted blood volume

[d] H=height in cm

hematocrit was below normal range in the majority of patients independent of the degree of splenic and hepatic enlargement Red cell mass was decreased in only 3 of 12 patients with marked hepatosplenomegaly whereas it was below the normal range in 10 of 22 children without considerably enlarged spleen and liver Accordingly the mean red cell mass of the patients with marked hepatosplenomegaly (?6 7±8 7 ml/kg) was significantly higher ($p <$ 0 025) than the mean value of the children without marked hepatosplenomegaly (20 8± 6 0 ml/kg) although the mean hematocrit values were similar in both groups (0 282± 0 078 and 0 295±0 083 respectively)

DISCUSSION

The calculation of total blood volume and red cell mass using measurement of plasma volume and hematocrit was based on the assumption of a constant body/venous hematocrit ratio The ratio of 0 91 used in our study has been found to be constant over a wide hematocrit range in newborn infants and adults (5 6) In adult patients the body/venous hematocrit ratio has been shown to increase in relation to spleen size (9 11 18) A similar relationship between the body/venous hematocrit ratio and spleen size has been found by our working group in a study on 7 children with acute lymphoblastic leukemia (15) The wide range of the data reported in the literature does not allow prediction of the true individual values We have therefore used the same ratio of 0 91 for all patients Provided that the body/venous hematocrit ratio in our splenomegalic patients was higher than 0 91 the blood volume in these patients was underestimated This implies an even greater hypervolemia in the patients with marked enlargement of the spleen as estimated

Increased blood volumes and plasma volumes due to splenomegaly have been found by other authors in adult patients with lymphoproliferative and myeloproliferative disorders (1–4 7 8 12 13 19–22) In these studies liver size has not been considered Our results indicate that blood volume in leukemic children is substantially influenced by spleen and liver size

Anemia is a predominant symptom in childhood leukemia It may be caused by splenic pooling of red blood cells shortened red cell survival time or depressed erythropoiesis (7 23) In hepatomegaly blood pooling in the liver and in the portal vein area may occur (14) According to our findings hemodilution due to increased plasma volume can be con

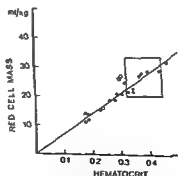


Fig 2 Relationship between red cell mass and hematocrit in patients with spleen plus liver sizes per height of more than 0 05 (O) and less than 0 05 cm/cm (●) Line represents relationship between red cell mass and hematocrit for a normal blood volume of 77 ml/kg (17) calculated as a product of normal blood volume hematocrit and the factor 0 91 The boxed area shows the normal range (±2 S D)

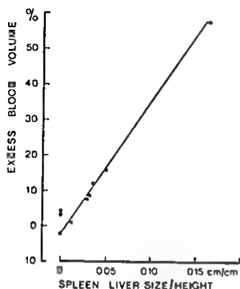


Fig 1 Relationship of blood volume expressed as a percentage of the predicted blood volume to spleen plus liver size related to height

tion of Lugol s solution before and for 5 days after injection of the radionuclide Normal blood volumes were derived from nomograms (17)

RESULTS

In 26 of the 34 patients the blood volume was greater than that predicted from nomograms for normal blood volumes In 15 children blood volume was found to be over 10% in excess of normal values (Fig 1) Table 1 summarizes the blood volume data in relation to the types of leukemia and to the stage of disease in the children with acute lymphoblastic leukemia Low red cell mass and hematocrit values were found in the children with acute lymphoblastic leukemia before treatment during induction therapy and in those with relapses Plasma volume and blood volume were considerably increased in the children with acute lymphoblastic leukemia during induction therapy and in the patients with various types of leukemia The majority of these children showed marked enlargement of the spleen and/or liver whereas only a few of the children with acute lymphoblastic leukemia in remission or with relapse had enlarged spleen or liver This indicates that hepatosplenomegaly was responsible for the hypervolemia

Correlations between excess blood volume (i e difference between measured and predicted blood volumes) and spleen and liver size are summarized in Table 2 The correlations were highly significant ($p<0\ 001$) The highest correlation coefficient was found when excess blood volume expressed as a percentage of the predicted blood volume was related to spleen plus liver size per cm of height ($r=0\ 85$) Fig 1 shows that blood volume increased linearly as spleen and liver size per cm of height increased

Fig 2 shows the relationship between red cell mass and hematocrit in the patients with marked hepatosplenomegaly and those with moderately or without enlarged spleen and liver In most cases the red cell mass of patients with marked hepatosplenomegaly was considerably higher than expected from their hematocrit Moreover Fig 2 illustrates that

Table 1 *Blood volume data in children with leukemia ($\bar{x}\pm$S D)*

Disorder stage	*n*	Age (years)	Hematocrit	Plasma volume (ml/kg)	Red cell mass (ml/kg)	Blood volume (ml/kg)
ALL						
Before treatment	9	1–11	0 287±0 068	63 0±13 5	21 6± 4 5	84 6±13 0
Induction therapy	4	9/12–14	0 226±0 079	77 9±24 9	19 5± 7 4	97 4±26 3
Remission	9	3–10	0 349±0 060	54 3± 6 6	25 2± 4 2	79 5± 5 5
Relapse	5	5–13	0 231±0 060	61 4± 7 6	16 3± 4 0	77 7± 7 2
AGL CGL AML	7	2–16	0 300±0 089	71 7± 7 0	28 3±11 4	100 0±16 1
Normal	93	9/12–14	0 396±0 042	49 8± 6 7	27 0± 3 6	76 8± 8 6

ALL=acute lymphoblastic leukemia AGL=acute granulocytic leukemia CGL=chronic granulocytic leukemia AML=acute monocytic leukemia

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LONG TERM EFFECT OF PREVIOUS SWIMTRAINING IN GIRLS A 10 YEAR FOLLOW UP OF THE GIRL SWIMMERS

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ABSTRACT Eriksson B O Engstrom I Karlberg P Lundin A Saltin B and Thoren C (Department of Paediatrics University of Goteborg and the Departments of Paediatrics Karolinska Institutet St Goran s Hospital and Karolinska Hospital Stockholm Sweden) Long term effect of previous swimtraining in girls A 10-year follow up of the girl swimmers Acta Paediatr Scand 67 285 1978 —Thirty girls studied in 1961 after 2 5 years of intensive swimtraining were the subject of a follow up for ten years When last examined seven and ten years after the original study all the girls had given up swimtraining The increased values for vital capacity observed in 1961 remained unchanged but residual volume functional residual capacity and total lung capacity showed small increases even after corrections for body growth Such increases are however normal in these years Heart volume which was high originally was found to be lower ten years later although mean values were still higher than normal The decrease seen from 1961 to 1971 could mainly be ascribed to a decrease in the subjects with the largest hearts originally Both total hemoglobin and blood volume decreased to normal values in relation to body size Maximal oxygen uptake though fell from 2 [illegible] l/min (51 4 ml/kg×min) to 2 18 l/min (36 4 ml/kg×min) ten years later It is suggested that the functional capacity of the cardiovascular system declined more markedly than its dimensions

KEY WORDS Blood lactate concentration blood volume girl swimmers growth heart volume lung volumes maximal oxygen uptake physical training total hemoglobin

Excellence in athletic performance is due to both constitutional factors and intense prolonged physical training The effect of such training on the human body has been the subject of many studies (for reference see 3) The question of whether physical training started before the end of growth can have a particular effect on the human body has also been debated this was one of the issues in our original 1961 study of 30 girls 12 to 16 years old who trained for competitive swimming (2)

We found increased values for blood and heart volumes vital capacity total lung capacity and total haemoglobin These changes in the respiratory and circulatory systems were closely correlated with one another and with an increased aerobic capacity indicating well controlled adaptation of the cardiovascular system to the strenuous physical training performed

The present study is a follow up of the same girls using the same physiological measurements 2 (1963) 4 (1965) 7 (1968) and 10 years (1971) after the original study

MATERIAL

At the 1963 examination only the nine girls belonging to club B the most successful club were studied All thirty girls were examined in 1965 and 1968 but two later moved abroad and were not included in 1971 (Table 1) At the

sidered as an additional factor decreasing hematocrit Evidently hematocrit reflects red cell mass only as long as spleen and liver are not enlarged It is therefore explicable that leukemic children despite low hematocrit values show little symptomatic evidence of apparent anemia On the other hand leukemic children with marked hepatosplenomegaly require higher volumes of transfused red blood cells when a certain increase in hematocrit is desired

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Table 2 *Mean values with ±S D for some physiological data for girl swimmers in five different studies 1961 1963 1965 (-66) 1968 (-69) and 1971 The number of girls in each study is indicated*

	1961	1963	1965 (–66)	1968 (–69)	1971
n	30	9	30	30	?8
Age years	12 9	17 0	18 4	21 4	23 9
Height cm	164 8±6 5	170 0±3 7	167 9±5 5	168 5±5 7	168 5±6 [illegible]
Weight kg	54 2±7 3	6? 5±8 1	58 7±8 0	[illegible] 9±6 4	59 6±6 1
TLC l BTPS	4 91±0 81	5 98±0 45	5 63±0 78	5 37+0 72	–
VC l BTPS	4 00±0 67	4 70±0 [illegible]	4 30±0 61	4 ?5±0 57	4 38±0 57
FRC l BTPS	? 04±0 40	? 60±0 37	2 58±0 45	? 51±0 50	–
RC l BTPS	0 95±0 [illegible]	1 29±0 18	1 34±0 31	1 11±0 37	–
HV ml	625±119	769±57	640±105	630±103	645±90
THb g	58?±98	621±6?	488±69	517±76	–
BV l	4 70±0 73	4 9 ±0 51	3 70±0 45	4 29±0 68	–
$\dot{V}_{O_2}$ max l/min STPD	2 80±0 44	3 14±0 2?	2 49±0 32	2 ?4±0 4?	? 18±0 31
$\dot{V}_E$ max l/min BTPS	99 9±16 5	105 6±5 4	89 5±15 3	[illegible] 4±20 0	90 6±22 3
HR beats/min	198 9±7 9	?00 9±6 1	197 7±8 4	195 6±8 6	193 7±9 4
Lactate mmol/l	11 76±? 33	10 10±1 36	11 55±? 08	10 37±2 34	1? 2?±2 39

RESULTS

Between 1961 and 1971 there was an increase in body size in the girls (Table 2) they increased by an average of 4 cm in height and 5 kg in weight As a result of this all the other variables studied have had to be corrected for growth (Table 3) Even after this correction vital capacity was found to be high at the time of the first study in 1961 (corrected to a height standard) and it did not subsequently increase any further (Fig 2) Residual volume functional residual capacity and (because vital capacity was stable) also TLC displayed significant increases compared with the values from 1961 (Table 3) Heart volume was 21 5% larger than the value predicted in the 1961 study and absolute values remained unchanged in the period from 1963 to 1971 A slight decrease not statistically significant was observed when the change in body size was taken into account Both total hemoglobin and blood volume were reduced by 16 3% and 14 4% respectively 1971 as compared to 1961 and were related to the girls work capacity in all parts of the study

Mean maximal oxygen uptake ($\dot{V}_{O_2}$ max) amounted to 2 80 l/min in the 1961 study (Table 2) and underwent a marked decrease

Table 3 *Mean values with ±S D for some physiological parameters corrected for the difference in body size for girl swimmers The degree of significances between values obtained in 1961 and the other years is included (***=p<0 001 **=p<0 01 *=p<0 05)*

	1961	1963	1965	1968	1971
n	30	9	30	30	28
BW/H³ kg/m³	12 08±0 98	1? 72±1 53	[illegible] 38±1 2?	1? ?9±0 88	12 46±1 05
TLC/H³ l/m	1 09±0 10	1 ?2±0 09	1 18±0 22	1 13±0 10	–
VC/H³ l/m	0 87±0 09	0 96±0 07	0 90±0 10	0 90±0 08	0 90±0 08
FRC/H l/m	0 45±0 06	0 53±0 07	0 54±0 07	0 52±0 08	–
RV/H l/m	0 21±0 05	0 ?7±0 03	0 28±0 06	0 23±0 08	–
HV/H³ ml/m	138 5±17 8	156 0±1 4	134 4±15 5	131 1±15 7	135 0±15 5
THb/H g/m	1?9 2±14 0	128 4±11 5	10? 0±8 7*	108 1±8 1	–
BV/H l/m³	1 04±0 1?	1 0?±0 10	0 77±0 06	0 89±0 08	–
$\dot{V}_{O_2}$ max/H³ l/min×m³	1 03±0 12	1 08±0 06	0 88±0 09	0 78±0 12	0 76±0 08 *
$\dot{V}_{O_2}$ max/BW ml/kg×min	51 54±4 35	51 14±5 91	42 09±4 40	37 94±5 49	36 41±3 21
$\dot{V}_E$ max/H³ l/min×m³	36 75±5 3?	36 54±1 93	31 94±5 08	31 8±? 2?	32 32±7 23

Table 1 *The number of girls studied in the different examinations In the 1965 study two girl were studied in the beginning of 1966 and in the 1968 study seven girls were studied in the be ginning of 1969*

Determination	1961	1963	1965 (and early 1966)	1968 (and early 1969)	1971
Number of girls examined	30	9	30	30	28
Training	30	8	5	0	0
Non training	0	1	25	30	30
Lung volumes					
TLC FRC RV	30	9	29	28	–
VC	30	9	29	28	25
Heart volume	30	8	30	30	28
THb and blood volume	30	9	29	30	–
Exercise tests	30	9	28	30	28

final examination (1971) the average age of the girls was 23 9 years (range 21 4–26 3) The average interval since the girls had stopped swimtraining was 7 5 years (Fig 1) the average age at cessation being 16 6 years (range 13 3–20 7) The girl who continued to train longer did so until the 1968 Olympic Games Eight of the nine girls studied in 1963 were still training then but by 1965 only five out of thirty were training *All had stopped competition train* ing by the time of the 1968 and 1971 studies (Table 1) During the years of training the intensity and amount of training did not differ from the values reported earlier (2) Seven of the nine girls in club B qualified for the Swedish team for the European Championship in 1962 and two also qualified for the 1964 Olympic Games One competed successfully in the 1966 European Championships and at the 1968 Olympic Games

After they had given up swimtraining most of the girls assumed a sedentary mode of life although a few of them did some downhill skiing jazz ballet dancing or calisthenics In 1971 eleven of the thirty girls were married one was divorced and another had married a second time Nine of them had had a total of twelve children Eleven had attained the equivalent of a junior college education (*studentexamen*) nine had completed high school studies five junior high school *(realexamen) and five elementary* school Almost all of them worked six as teachers five nurses four secretaries three air stewardesses and four still at college Only the girl who had married and was living abroad did not have a job

METHODS AND PROCEDURE

With a few exceptions all the methods used in the examinations in 1963 1965 and 1968 were the same as those used in 1961 In 1971 however only measurements of vital capacity heart volume and exercise testing were performed

Total lung capacity (TLC) and its subdivisions were measured by the closed circuit helium dilution technique (7 10) and vital capacity (VC) using a Bernstein spirometer (7 10) Heart volume (HV) was determined fron biplanar radiographs with the subject in a recumben position (18) total hemoglobin (THb) by the alveolar CO method (21) and blood volume (BV) was calculated fron THb and the hemoglobin concentration of blood drawi from the fingertip

Exercise testing was performed on a Krogh bicycl ergometer at a constant *pedalling rate of 50 rpm excep* in the 1971 study when an Elema electrically braked ergo meter was used at a rate of 60 rpm Exercise was per formed at two or three submaximal loads before the maxi mal load was used At least two maximal exercise test were performed on different days in each case Th leveling off criterion was used to establish maximal oxy gen uptake (3) Expired gas was collected in Douglas bags its volume measured in a balanced Tissot spirometer an gas analyses performed using a modified Haldane tech nique

Heart rate was measured from ECG recordings Bloo lactate concentrations were determined on blood drawi from a pre warmed fingertip and analysed by a colori metric technique (4 22)

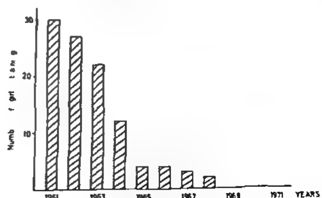


Fig 1 The number of girls still training at the beginning of each year during the period from the first study in 1961 to the last study in 1971

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	1961	1963	1965 (-66)	1968 (-69)	1971
	30	9	30	30	28
Age years	1? 9	17 0	18 4	21 4	23 9
Height cm	164 8±6 5	170 0±3 7	167 9±5 5	168 5±5 7	168 5±6 0
Weight kg	54 2±7 3	6? 5±8 1	58 7±8 [illegible]	58 9±6 4	59 6±6 1
TLC l BTPS	4 91±0 81	5 98±0 45	5 63±0 78	5 37±0 72	–
VC l BTPS	4 00±0 67	4 70±0 31	4 30±0 61	4 25±0 57	4 38±0 57
FRC l BTPS	? 04±0 40	2 60±0 37	? 58±0 45	2 51±0 50	–
RC l BTPS	0 95±0 8	1 ?9±0 18	1 34±0 31	1 11±0 37	–
HV ml	625±119	769±57	640±105	630±103	645±90
THb g	582±98	6?1±6?	488±69	517±76	–
BV l	4 70±0 73	4 9_±0 51	3 70±0 45	4 29±0 68	–
$\dot{V}_{O_2}$ max l/min STPD	2 80±0 44	3 14±0 2?	? 49±0 3?	2 24±0 42	2 18±0 31
$\dot{V}_E$ max l/min BTPS	99 9±16 5	105 6±5 4	89 5±15 3	88 4±?0 0	90 6±?2 3
HR_m beats/min	198 9±7 9	200 9±6 1	197 7±8 4	195 6±8 6	193 7±9 4
$Lactate_m$ mmol/l	11 76±? 33	10 10±1 36	[illegible] 55±? 08	10 37±2 34	12 2?±2 39

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	1961	1963	1965	1968	1971
n	30	9	30	30	[illegible]
BW/H kg/m	1? 08±0 98	12 7 ±1 53	12 38±1 22	12 ?9±0 88	12 46±1 05
TLC/H³ l/m	1 09±0 10	1 ??±0 09	1 18±0 2?	1 13±0 10	–
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RV/H³ l/m	0 ?1±0 05	0 27±0 03	0 28±0 06	0 23±0 08	–
HV/H ml/m³	138 5±17 8	156 0±1 4	134 4±15 5	131 1±15 7	135 0±15 5
THb/H g/m³	1?9 ±14 0	1.8 4±11 5	10? 0±8 7	108 1±8 1	–
BV/H l/m	1 04±0 12	1 0?±0 10	0 77±0 06	0 89±0 08	–
$\dot{V}_{O_2}$ max/H³ l/min×m³	1 03±0 1?	1 08±0 06	0 88±0 09	0 78±0 1?	0 76±0 08
$\dot{V}_{O_2}$ max/BW ml/kg×min	51 54±4 35	51 14±5 91	42 09±4 40	37 94±5 49	36 41±3 21
$\dot{V}_E$ max/H³ l/min×m³	36 75±5 3?	36 54±1 93	31 94±5 08	31 ?8±7 2?	32 32±7 23

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TLC FRC RV	30	9	29	28	–
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Total lung capacity (TLC) and its subdivisions were measured by the closed circuit helium dilution technique (7 10) and vital capacity (VC) using a Bernstein spirometer (7 10) Heart volume (HV) was determined from biplanar radiographs with the subject in a recumbent position (18) total hemoglobin (THb) by the alveolar CO method (21) and blood volume (BV) was calculated from THb and the hemoglobin concentration of blood drawn from the fingertip

Exercise testing was performed on a Krogh bicycle ergometer at a constant pedalling rate of 50 rpm except in the 1971 study when an Elema electrically braked ergometer was used at a rate of 60 rpm Exercise was performed at two or three submaximal loads before the maximal load was used At least two maximal exercise tests were performed on different days in each case The leveling off criterion was used to establish maximal oxygen uptake (3) Expired gas was collected in Douglas bags its volume measured in a balanced Tissot spirometer and gas analyses performed using a modified Haldane technique

Heart rate was measured from ECG recordings Blood lactate concentrations were determined on blood drawn from a pre warmed fingertip and analysed by a colorimetric technique (4 22)

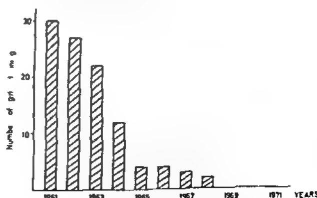


Fig 1 The number of girls still training at the beginning of each year during the period from the first study in 1961 to the last study in 1971

Table 2 *Mean values with ±S D for some physiological data for girl swimmers in five different studies 1961 1963 1965 (-66) 1968 (-69) and 1971 The number of girls in each study is indicated*

	1961	1963	1965 (–66)	1968 (–69)	1971
n	30	9	30	30	28
Age years	1 9	17 0	18 4	21 4	23 9
Height cm	164 8±6 5	170 0±3 7	167 9±5 5	168 5±5 7	168 5±6 0
Weight kg	54 ?±7 3	62 5±8 1	58 7±8 0	58 9±6 4	59 6±6 1
TLC l BTPS	4 91±0 81	5 98±0 45	5 63±0 78	5 37±0 7?	–
VC l BTPS	4 00±0 67	4 70±0 31	4 30±0 61	4 25±0 57	4 38±0 57
FRC l BTPS	2 04±0 40	? 60±0 37	2 58±0 45	2 51±0 50	–
RC l BTPS	0 95±0 8	1 29±0 18	1 34±0 31	1 11±0 37	–
HV ml	6?5±119	769±57	640±105	630±103	645±90
THb g	58?±98	621±6?	488±69	517±76	–
BV l	4 70±0 73	4 9?±0 51	3 70±0 45	4 29±0 68	–
$\dot{V}_{O_2}$ max l/min STPD	? [illegible]±0 44	3 14±0 2	? 49±0 3?	? 24±0 42	? 18±0 31
$\dot{V}_E$ max l/min BTPS	99 9±16 5	105 6±5 4	89 5±15 3	[illegible] 4±?0 0	90 6±?2 3
HR_m beats/min	198 9±7 9	00 9±6 1	197 7±8 4	195 6±8 6	193 7±9 4
$Lactate_m$ mmol/l	11 76±2 33	[illegible] 10±1 36	11 55±? 08	10 37±2 34	1? 22±2 39

RESULTS

Between 1961 and 1971 there was an increase in body size in the girls (Table 2) they increased by an average of 4 cm in height and 5 kg in weight As a result of this all the other variables studied have had to be corrected for growth (Table 3) Even after this correction vital capacity was found to be high at the time of the first study in 1961 (corrected to a height standard) and it did not subsequently increase any further (Fig 2) Residual volume functional residual capacity and (because vital capacity was stable) also TLC displayed significant increases compared with the values from 1961 (Table 3) Heart volume was 21 5% larger than the value predicted [illegible] the 1961 study and absolute values remained unchanged in the period from 1963 to 1971 A slight decrease not statistically significant was observed when the change in body size was taken into account Both total hemoglobin and blood volume were reduced by 16 3% and 14 4% respectively 1971 as compared to 1961 and were related to the girls work capacity in all parts of the study

Mean maximal oxygen uptake ($\dot{V}_{O_2}$ max) amounted to 2 80 l/min in the 1961 study (Table 2) and underwent a marked decrease

Table 3 *Mean values with ±S D for some physiological parameters corrected for the difference in body size for girl swimmers The degree of significances between values obtained in 1961 and the other years is included (***=p<0 001 **=p<0 01 *=p<0 05)*

	1961	1963	1965	1968	1971
n	30	9	30	30	28
BW/H kg/m	1? 08±0 98	12 72±1 53	12 38±1 2?	1? ?9±0 88	12 46±1 05
TLC/H³ l/m	1 09±0 10	1 ?2±0 09	1 18±0 2?	1 13±0 10	–
VC/H³ l/m³	0 87±0 09	0 96±0 07	0 90±0 10	0 90±0 08	0 90±0 08
FRC/H³ l/m	0 45±0 06	0 53±0 07	0 54±0 07	0 5?±0 08	–
RV/H l/m	0 ?1±0 05	0 ?7±0 03	0 ?8±0 06	0 23±0 08	–
HV/H ml/m	138 5±17 8	156 0±12 4	134 4±15 5	131 1±15 7	135 0±15 5
THb/H g/m³	1?9 2±14 0	128 4±11 5	10? 0±8 7	108 1±8 1	–
BV/H l/m	1 04±0 1?	1 0 ±0 10	0 77±0 06	0 89±0 08	–
$\dot{V}_{O_2}$ max/H³ l/min×m³	1 03±0 1?	1 08±0 06	0 88±0 09	0 78±0 12	0 76±0 08
$\dot{V}_{O_2}$ max/BW ml/kg×min	51 54±4 35	51 14±5 91	4? 09±4 40	37 94±5 49	36 41±3 21
$\dot{V}_E$ max/H³ l/min×m³	36 75±5 3	36 54±1 93	31 94±5 08	31 28±7 22	32 32±7 23

Table 1 *The number of girls studied in the different examinations In the 1965 study two girls were studied in the beginning of 1966 and in the 1968 study seven girls were studied in the beginning of 1969*

Determination	1961	1963	1965 (and early 1966)	1968 (and early 1969)	1971
Number of girls examined	30	9	30	30	28
Training	30	8	5	0	0
Non training	0	1	25	30	30
Lung volumes					
TLC FRC RV	30	9	29	28	–
VC	30	9	29	28	25
Heart volume	30	8	30	30	28
THb and blood volume	30	9	29	30	–
Exercise tests	30	9	28	30	[illegible]

final examination (1971) the average age of the girls was 23 9 years (range 21 4–26 3) The average interval since the girls had stopped swimtraining was 7 5 years (Fig 1) the average age at cessation being 16 6 years (range 13 3–20 7) The girl who continued to train longer did so until the 1968 Olympic Games Eight of the nine girls studied in 1963 were still training then but by 1965 only five out of thirty were training All had stopped competition training by the time of the 1968 and 1971 studies (Table 1) During the years of training the intensity and amount of training did not differ from the values reported earlier (2) Seven of the nine girls in club B qualified for the Swedish team for the European Championship in 1962 and two also qualified for the 1964 Olympic Games One competed successfully at the 1966 European Championships and at the 1968 Olympic Games

After they had given up swimtraining most of the girls assumed a sedentary mode of life although a few of them did some downhill skiing jazz ballet dancing or calisthenics In 1971 eleven of the thirty girls were married one was divorced and another had married a second time Nine of them had had a total of twelve children Eleven had attained the equivalent of a junior college education (*studentexamen*) nine had completed high school studies five junior high school (*realexamen*) and five elementary school Almost all of them worked six as teachers five nurses four secretaries three air stewardesses and four still at college Only the girl who had married and was living abroad did not have a job

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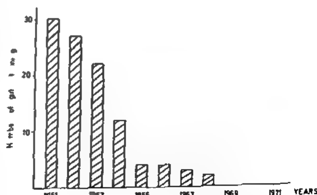


Fig 1 The number of girls still training at the beginning of each year during the period from the first study in 1961 to the last study in 1971

a		
b	Club A C D	Diff B/A C D
7	167 [illegible]	n s
6	57 6	
[illegible]	1[illegible] 3	n s
	–	–
95	0 89	n s
	–	–
	–	–
6	134 7	n s
	–	–
	–	–
6	30 6	
44	[illegible] 01	
83	0 74	
8	35 9	n s
1	19 8	n s
9	1[illegible] 0	n s

order to assess the differences produced by different training intensities and attained performances (Table 4 and Fig 3) In the 1961 study the nine girls had higher mean values for vital capacity but no significant differences were found for TLC FRC or residual volume In the ten year follow up period no changes were found in the different lung volumes whether they were related to height cubed or as differences between the best girls and the others It would thus appear that the question of whether the swimtraining produced increased lung volumes initially or girls with constitutionally bigger lungs became top swimmers remains open In another longitudinal study of 29 girls followed from the age of 10–12 to 16 years we found that the girls who continued training throughout the follow up period increased their lung volumes (that is their vital capacity) *more than could be anticipated from growth alone whereas the girls* who stopped training early failed to display this increase (9) Increased lung volumes were found in 18 boys aged 10 years who had just started swimtraining so that large lungs may be a requirement for becoming a top swimmer (12) Because of these results it is perhaps best to conclude that both constitution and training are important factors in the making of a top swimmer

In marked contrast to the changes in lung volumes total hemoglobin decreased over the follow up with blood volume falling slightly less (Fig 3) The girls with the highest values for these two variables in 1961 also showed greater declines than the others (Table 4 and Fig 3) so that the highly significant differences found between the girls in club B and the others in 1961 had almost disappeared by the 1965 and 1968 studies

Both active and retired top athletes have increased heart volumes (6 16 17 20) and older but active athletes show a stroke volume during exercise that is related to heart volume (16) This relationship could not be found in these girl swimmers (13) so that there may be some impairment in heart performance Although no other signs of impaired heart function could be found it would seem necessary to undertake a longer term follow up for another 30 to 50 years to evaluate this possibility An increased heart volume as such must not necessarily be indicative of heart abnormalities and it may undergo long term changes In 1961 a significant difference was found between the heart volumes of the girls from club B (150 7 ml/m³) and the other girls (133 3 ml/m³) This difference had disappeared by 1971 when the figures were 135 [illegible] ml/m³ against 134 7 ml/m³ (Fig 3) The girls with the largest hearts thus displayed a decrease *in* heart volume whilst the heart volumes of the other subjects remained unchanged This decrease in heart volume in the top swimmers may be taken as an indicator of a partial adaptation to a non training situation in a way less pronounced than that of total hemoglobin blood volume and aerobic power

The remarkable decrease in V_O found *in* this study exceeds values reported previously (for reference see 3) to such an extent that it is only comparable with the decrease found after several weeks of bed rest (19) When in

Table 4 *Mean values for some physiological parameters ın nıne gırls from club B and ın 21 gr from clubs A+C+D ın the 1961, 1965 1968 and 1971 studıes Also ıncluded are the levels of signi cance for dıfferences between the gırls ın club B as compared to the other 21 gırls*

	1961			1965			1968		
	Club B	Club A+C+D	Diff B/A C D	Club B	Club A C D	Diff B/A C D	Club B	Club A C D	Diff B/A C D
Height cm	167 6	163 6	n s	170 2	167 0	n s	171 3	167 3	n s
BW kg	57 8	52 7	n s	60 81	57 9	n s	62 4	57 4	*
BW/H³ kg/m³	12 2	12 0	n s	12 3	12 4	n s	12 4	1? 2	n s
TLC/H³ l/m³ BTPS	1 13	1 01	n s	1 25	1 15	*	1 19	1 11	n s
VC/H³ l/m³ BTPS	0 93	0 87	*	0 96	0 87	*	0 95	0 88	
FRC/H³ l/m³ BTPS	0 47	0 45	n s	0 56	0 53	n s	0 53	0 52	n s
RV/H³ l/m³ BTPS	0 20	0 22	n s	0 28	0 28	n s	0 24	0 23	n s
HV/H³ ml/m³	150 7	133 3	*	144 3	130 2	*	139 7	127 5	*
THb/H³ g/m³	142 8	123 4	**	104 3	100 9	n s	112 2	106 3	n s
BV/H³ l/m³	1 16	0 99	***	0 78	0 77	n s	0 94	0 87	
V_E max/H³ l/min ×m³ BTPS	40 5	35 2	**	33 4	31 2	n s	34 9	29 7	n s
V_O max l/min STPD	3 18	2 63		2 66	2 40	*	2 53	2 11	*
V_O max/H³ l/min×m³	1 13	0 99	***	0 92	0 86	n s	0 86	0 75	*
V_O max/BW ml/kg×min	55 1	50 0	**	43 9	41 2	n s	40 7	36 3	n s
HR_{max} beats/min	202 3	197 4	n s	199 6	196 8	n s	198 8	194 2	n s
$Lactate_m$ mmol/l	11 8	11 7	n s	11 1	11 7	n s	10 5	10 3	n s

ın 1965 (2 49 l/mın) 1968 (2 24 l/mın) and 1971 (2 18 l/mın) Whether these are expressed per kg of body weight or per m height squared (Table 3) thıs decrease ıs seen to be more pronounced ın relatıon to the change ın body sıze thus the 1961 values of 51 4 ml/kg×mın (range 58 0–42 5) had decreased to 36 4 ml/kg ×mın (range 41 7–30 2) ın the 1971 study

Maxımal ventılatıon (V_E max) also decreased (Table 2) although not to the same extent as the fall ın V_O max Thus the ventılatory coeffıcıent ın maxımal exercıse (V_E max/V_O max) ıncreased from 35 7 ın 1961 to 35 9 39 5 and 41 6 ın the 1965 1968 and 1971 studıes respectıvely In addıtıon mean maxımal heart rate decreased from 199/mın to 194/mın between the 1961 and 1971 studıes although maxımal blood lactate concentratıon remaıned relatıvely unchanged

DISCUSSION

In 1961 the group of thırty subjects was taller on average than a Swedısh growth standard (2) and theır ıncrease ın heıght was apparently more pronounced durıng the swımtraınıng perıod of theır lıves (2) However as was previously reported (15) thıs further heıght ıncrease was dırectly and wholly attrıbutable to theır early attaınment of puberty In vıew of thıs theır heıght ın the 1971 study could be predıcted from that at the age of seven Sımılarly body weıght had not ıncreased more than that whıch could be predıcted from heıght (Table 3) although a few gırls showed a hıgh ınıtıal weıght gaın durıng the fırst few years after gıvıng up traınıng The weıght subsequently decreased to values wıthın normal lımıts

The fırst study also showed that vıtal capacıty had ıncreased ın relatıon to heıght standard (2) and a further ıncrease ın absolute values was obtaıned over the years of thıs work However thıs ıncrease was dırectly related to the growth ın body sıze (Table 3) unlıke resıdual volume (FRC and TLC) whıch ıncreased more than could be ascrıbed to normal growth In spıte of thıs both resıdual volume and FRC values fall wıthın one standard devıatıon on eıther sıde of the normal Swedısh standards (5 10) we therefore made a separate analysıs of the nıne gırls from club B ın

971		
lub	Club A C D	Diff B/A C D
71 7	167 [illegible]	n s
64 6	57 6	
1 8	1[illegible] 3	n s
	–	–
0 95	0 89	n s
	–	–
–	–	–
35 6	134 7	n s
–	–	–
	–	–
36 6	30 6	
[illegible] 44	[illegible] 01	
0 83	0 74	
37 8	35 9	n s
95 1	[illegible] 8	n s
1[illegible] 9	1[illegible] 0	n s

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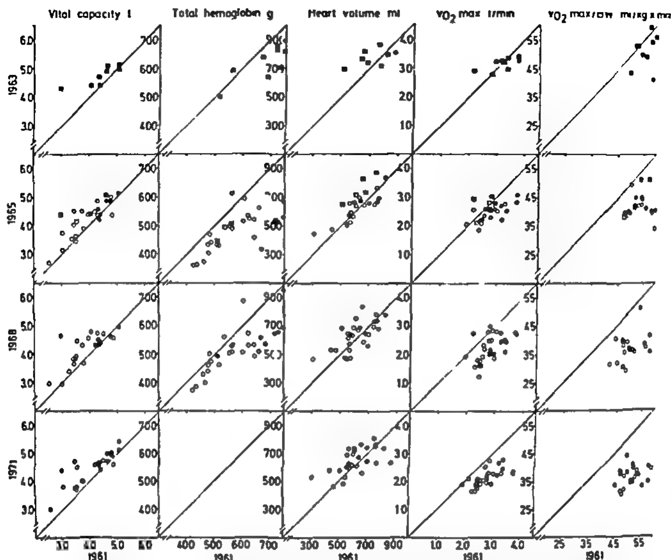


Fig 2 Individual values for vital capacity total hemoglobin heart volume maximal oxygen uptake and maximal oxygen uptake per kg of body weight in 30 girl swimmers at the studies in 1963 1965 1968 and 1971 as compared to the 1961 study The 45 line of indentity is drawn Bold symbols denote the nine girls in club B and open symbols the other 21 Squares indicate that the girls were still training at the time of the examination and circles indicate that they had stopped training

creases in body size were taken into account the fall amounted to some 30% (related to kg body weight) and 26% (related to body height squared) The nine best girls who had significantly higher values than the other subjects in 1961 retained their difference over the others regardless of whether body size was taken into account or not (Table 4 and Fig 4) However the decrease was more pronounced in the top girls and their greater height and weight combined to make corrected values less different from the others than absolute values

In the 1961 study a good correlation was found between the increased heart volume and the increased V_{O_2} max ($r=0$ 90) This correlation declined gradually 1965 $r=0$ 68 1968 $r=0$ 76 and 1971 $r=0$ 56 (Fig 5) The good correlation between heart volume and aerobic power was taken as a criterion of normality in the 1961 study If this is true the situation in *1971 must be regarded as less normal* However as discussed above no definite conclusion can be drawn on the basis of these results

In the initial study maximal blood lactate concentration values were found to be increased This may be of special importance in children in that they have a lower anaerobic

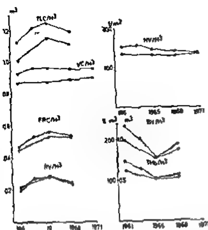


Fig 3 Mean values for total lung capacity (*TLC*) vital capacity (*VC*) functional residual capacity (*FRC*) residual volume (*RV*) heart volume (*HV*) blood volume (*BV*) and total hemoglobin (*THb*) corrected for the differences in body sizes in former girl swimmers at the studies in 1961 1963 1965 1968 and 1971 Bold circles denote the nine girls from club B and open circles the other 21 girls The shadowed areas indicate ±1 S D for Swedish people (5 10 [redacted] 21)

lactacid capacity than adults (1 11) and it could have been expected that the lactate value would fall on cessation of training However [redacted] these groups increasing age is associated with increasing maximal blood lactate

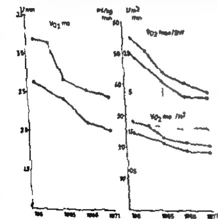


Fig 4 Mean values for maximal oxygen uptake (Vo_2 max) maximal oxygen uptake per height squared (Vo_2 max/BW) in former girl swimmers at the studies in 1961 1963 1965 1968 and 1971 For an explanation of symbols see Fig 3 The two shadowed areas for Vo_2 max/BW indicates the normal values with *±1 S D* for Swedish people given by P -O Åstrand (1) and I Åstrand (3)

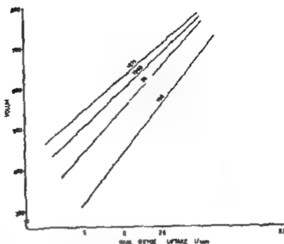


Fig 5 The regression lines for heart volume in relation to maximal oxygen uptake at the studies in 1961 1965 1968 and 1971 in former girl swimmers

concentration (14) so that the apparent lack of change in this value may fit current ideas

In many respects the dimensions and physiology of the oxygen transport system in these swimmers were different from those of top athletes and ordinary sedentary people although the girls were quite normal in other respects This was also true for their social and educational development Clearly additional studies lasting a further ten to thirty years will be necessary in order to evaluate the effects of intensive physical training on a growing subject

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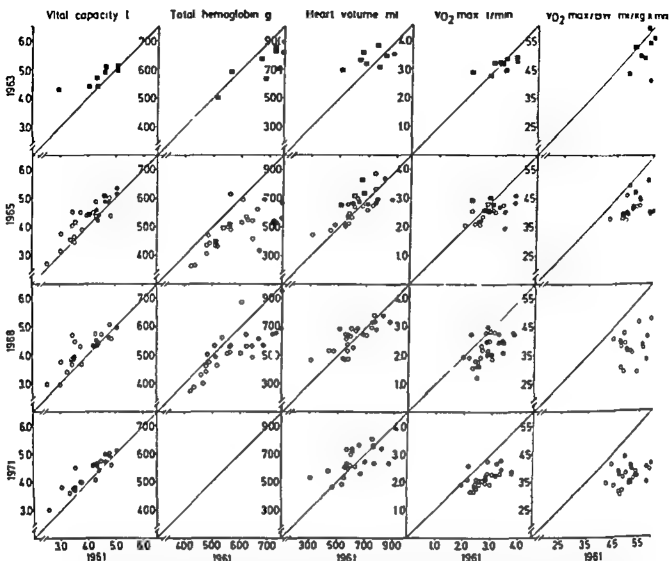


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SUCCESSFUL TREATMENT WITH CPAP OF TWO INFANTS WITH BRONCHOMALACIA

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ABSTRACT Neijens H J Kerrebijn K F and Smalhout B (Department of Paediatrics Subdepartment of Respiratory Diseases Erasmus University Medical School Sophia Children s Hospital Rotterdam and Institute of Anaesthesiology State University Utrecht the Netherlands) Successful treatment with CPAP of two infants with bronchomalacia Acta Paediatr Scand 67 293 1978 —Two children with severe bronchial collapse secondary to bronchomalacia improved dramatically after institution of continuous positive airway pressure (CPAP) Treatment was discontinued after 14 weeks without reappearance of symptoms Repeated bronchoscopy revealed a diminution in the bronchial collapsibility It is suggested that CPAP should be given if generalised bronchomalacia is present to tide the children over a bad period while the bronchus is becoming more stable

KEY WORDS Bronchomalacia CPAP

In infants with dyspnoea and cough the chest X ray sometimes reveals one or more lobes to be overfilled with air compressing neighbouring lobes and sometimes displacing the mediastinum This syndrome is often described as congenital lobar emphysema (CLE) (1 5 8 16) At bronchography collapse of the bronchus of the affected lobe can be seen In a number of patients bronchoscopy reveals bronchial collapse in expiration This indicates that diminished rigidity of the bronchial wall exists in these patients (bronchomalacia) It has not always been possible in CLE to assign a particular mechanism Deficiency of bronchial cartilage has been demonstrated with variable frequency (4 10 12)

In 1969 Campbell (7) showed a diminished amount of cartilage in the bronchial wall of 12 children with CLE using the sensitive technique of microdissection and staining The treatment consists mostly of resection of the affected lobe (13 14 17) The course is often unfavourable in children in whom the bronchomalacia affects large parts of the bronchial tree In generalised bronchomalacia the symptoms will continue after resection We herewith describe the successful treatment of two infants with generalised bronchomalacia with continuous positive airway pressure (CPAP) CPAP was given for a number of weeks to prevent the bronchi from collapsing and to tide the children over until the bronchi became more rigid

CASE HISTORIES

H de K was born after a normal pregnancy and labour with a birth weight of 3 080 g Since birth he coughed and was dyspnoeic after drinking or crying he also had cyanotic spells and failure to thrive The chest X ray showed *general hyperinflation* shadowing in the right upper lobe mediastinal displacement to the right and narrowing of the trachea (Fig 1*a*) Bronchoscopy revealed almost complete collapse of the left and a decrease in the diameter of the right main bronchus (to about ½ its inspiratory diameter) during expiration

After naso tracheal intubation and CPAP (*5 cm H O*) the dyspnoea and cyanotic attacks disappeared immediately and there was a rapid improvement in the general condition During the intubation period atelectases occurred several times This was treated with intensive physiotherapy and antibiotics CPAP could be tapered off

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Fig 2 Bronchography of the second patient (E van B) reveals in almost complete block of the right middle lobe bronchus

bronchial wall abnormality (20) If the amount of bronchial cartilage is diminished as in the patients described by Campbell (7) MacMahon & Ruggieri (15) and Fischer et al (9) the airways will be more collapsible at an increased transpulmonary pressure such as during expiration or crying It is possible that the amount of cartilage is sufficient but that the rigidity is subnormal because of abnormal composition of the cartilage Due to narrowing of the bronchus the discharge of mucus can be impaired This predisposes to bronchopulmonary infections which in turn cause an increase in mucus production Thus a vicious circle is set up

Adults with emphysema and an increased collapsibility of their bronchi tend to breathe out through pursed lips (11) By this mechanism they increase their intrabronchial pressure and diminish collapsibility In infants with bronchomalacia the collapsibility of the bronchi is also increased Therefore we decided to treat the two patients described with CPAP in order to increase their intrabronchial pressure and to tide them over until the involved bronchi became more rigid That this possibility exists is supported by clinical observations in children with tracheomalacia and by the study of Sinclair Smith et al (18) He showed that the cartilage fragments of the right middle lobe

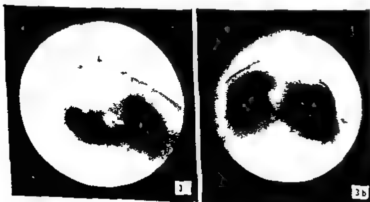


Fig 3 Bronchoscopic picture of carina and both main bronchi during expiration (*a*) before and (*b*) after 14 weeks of treatment with CPAP of the second patient

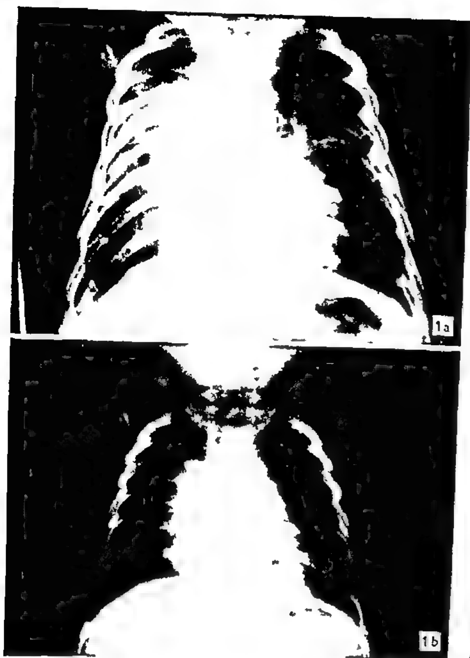


Fig 1 Chest X ray of the first patient (H de K) (*a*) before and (*b*) after 13 weeks of treatment Figure *a* shows hyperinflation shadowing of the right upper lobe and mediastinal displacement to the right Figure *b* is normal

over 1 week after 13 weeks of intubation The X ray showed a normal picture (Fig 1*b*) Although the child still has periods of cough and sputum production he has never had any further wheezing dyspnoea or cyanotic attacks He is now 4 years of age and his physical and mental development are unimpaired

E v B was born after a normal pregnancy and labour His birth weight was 4 000 g Shortly after birth he became dyspnoeic with severe exacerbations and copious sputum At the age of 2 months he was seen at the Wilhelmina Children s Hospital in Utrecht He had intercostal retraction in inspiration and rales could be heard over both lungs The chest X ray showed hyperinflation of the right middle lobe and compression of the right upper and lower lobes Bronchography revealed an almost complete block of the right middle lobe bronchus (Fig 2)

Bronchoscopy performed by one of us (B S) revealed narrowing of both main bronchi during expiration (Fig 3*a*) He was treated with antibiotics bronchodilators mucolytics and intermittent positive pressure ventilation without success The right middle lobe was resected at the age of 4 months In spite of the surgical intervention the child remained dyspnoeic and his general condition worsened Therefore he was intubated by nasotracheal tube and treated with CPAP (5 cm H_2O) which was highly successful CPAP could be stopped after 14 weeks At bronchoscopy it could be seen that the main bronchi were now wide open and not compressed during expiration (Fig 3*b*)

DISCUSSION

The severity of the symptoms in children with bronchomalacia depends on the extent of the



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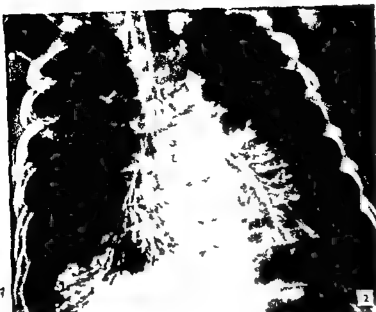


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In spite of intensive medical treatment and in the second child resection of the middle lobe symptoms were increasing until CPAP was given It thus seems highly probable that CPAP has been life saving

Although pneumothorax is a possible complication of CPAP therapy this was not seen in our two patients We had problems with nasal prongs for CPAP treatment in these children because of mouth breathing Therefore we used tracheal intubation Possibly these difficulties can be avoided using a facechamber as recently described (2) The children did not show laryngeal damage To prevent this we always use a polyethylene tube according to Berkovits (3) Verhoog Bloembergen & Leader (19) which is siliconised on both surfaces

The tube is changed once a week In our experience damage to the laryngeal mucosa is minimal with this regimen The impairment of mucus discharge causes a risk of atelectasis infection and tube occlusion Therefore regular suction physiotherapy and nursing in a specialized intensive care unit is necessary for successful and uncomplicated longterm intubation

ADDENDUM

After completion of this paper Campbell published a single case report of the use of CPAP in this disease (6)

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EFFECT OF INTRAVENOUS L ALANINE ADMINISTRATION ON PLASMA GLUCOSE INSULIN AND GLUCAGON BLOOD PYRUVATE LACTATE AND BETA HYDROXYBUTYRATE CONCENTRATIONS IN NEWBORN INFANTS

Study in Term and Preterm Newborn Infants

L SANN A RUITTON M MATHIEU J BOURGEOIS and J GENOUD

From the Department of Neonatology Inserm U 34 and the Laboratory of Enzymology Hopital Debrousse Lyon France

ABSTRACT Sann L Ruitton A Mathieu M Bourgeois J and Genoud J (Department of Neonatology Inserm U 34 and Laboratory of Enzymology Hopital Debrousse Lyon France) Effect of intravenous L alanine administration on plasma glucose insulin and glucagon blood pyruvate lactate and beta hydroxybutyrate concentrations in newborn infants Study III term and preterm infants Acta Paediatr Scand 67 297 1978.—Ten term and eleven preterm newborn infants with appropriate weights for their gestational age were infused for one minute with L alanine (150 mg/kg) at the age of 29 to 76 hours (mean 48 hours) and circulating levels of glucose lactate pyruvate D-betahydroxybutyrate (D-BOHB) insulin and glucagon were monitored Plasma glucose concentrations increased from 2 7±0 16 (mean±S E M) to 3 7±0 2 mmol/l after 50 min (p<0 01) in term infants In preterm infants after an initial decrease of the glucose level from 3 1±0 16 to 2 6±0 16 mmol/l (p<0 05) it returned to the baseline level at 50 min 3 0±0 2 mmol/l The blood concentration of D-BOHB decreased in term infants from 192±37 to 112±6 μM/l (p<0 01) after 40 min In preterms its decrease was not significant (p>0 05) Plasma glucagon levels rose from 53±5 to 70±8 pmol/l after ten minutes (p<0 01) in term infants and from 61±6 to 75±9 after 20 min (p<0 01) in preterm infants There were no significant changes in plasma insulin concentrations in either group Forty minutes after L alanine infusion I/G ratios were lower in preterm infants (1 26±0 14) than in term infants (1 71±0 25) (p<0 01) There was no relationship between the glycemic responses to L alanine and the basal levels of D-BOHB

The data suggest that the glycemic effect of L-alanine infusion and circulating glucagon depends upon a specific stage in maturation The antiketogenic effect of L alanine infusion is observed in term infants as in adults

KEY WORDS Newborn infants L-alanine plasma glucose insulin glucagon blood pyruvate lactate β-hydroxybutyrate

Glucose and D betahydroxybutyrate (D-BOHB) are major fuels for the fetal and neonatal brain (2 23) Both are influenced by alanine which plays a key role in the intermediary metabolism In adults intravenous or oral alanine administration results in an increase in plasma glucose concentration (7 17) and a drop of blood D-BOHB levels (11) In newborn infants the effect of oral or intravenous L alanine on plasma glucose concentrations has been studied (4 9 16 25 30 31) but the role of fetal maturation has not been analysed Furthermore the antiketogenic effect of alanine has not been investigated in newborn infants

The present study reports the results concerning the effect of intravenous L alanine administration on circulating levels of glucose

of normal children increase in number until some months after birth We could see by repeated bronchoscopy in the second child that the bronchial wall became more stable (Fig 3) The children have been intubated for 14 and 15 consecutive weeks respectively Both showed a clinical improvement as soon as CPAP was started and neither of them had any dyspnoea or wheezing after discontinuation of the treatment

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Table 2 *Effect of intravenous administration of L alanine (150 mg/kg) on blood pyruvate and lactate concentrations*

		Time (min)					
	No	■	10	∠0	30	40	50
Pyruvate (mM/l)							
Term infants	5	0 10±0 03	0 09±0 0?	0 10±0 03	0 09±0 03	0 05±0 01	0 14±0 07
Preterm infants	8	0 09±0 01	0 06±0 01	0 07±0 01	0 09±0 01	0 09±0 02	0 09±0 0?
Lactate (mM/l)							
Term infants	6	2 ?6±0 8	2 46±0 8	? 56±0 9	2 90±1 1	? 91±1 1	? 3?±0 8
Preterm infants	8	? 36±0 2	2 05±0 1	2 61±0 4	2 95±0 6	3 01±0 5	3 22±0 5

no significant change in plasma insulin (Table 3) There was no significant change of insulin/glucagon molar ratio and of blood pyruvate and lactate levels (Table 2) The blood concentration of D BOHB decreased after 10 min ($p<0\ 05$) and remained under the baseline level ($p<0\ 01$) (Fig 2)

In *premature infants* plasma glucose decreased after alanine administration ($p<0\ 05$) and returned to the baseline level (Fig 1) The plasma glucagon concentration rose significantly ($p<0\ 05$) but no change of plasma insulin was detected (Table 3) Insulin/glucagon molar ratio remained similar to the base line value There was no changes of blood pyruvate lactate (Table 2) and D-BOHB levels ($p>0\ 05$) (Fig 2)

Comparison between the two groups The basal levels of plasma glucose insulin and glucagon blood pyruvate and lactate were similar in both groups ($p>0\ 05$) The baseline levels of D-BOHB were however lower in preterm than in term infants ($p<0\ 05$) After alanine administration plasma glucose and blood D-BOHB were lower in preterm infants There was no difference for the lactate and pyruvate levels the insulin and glucagon concentrations After 40 min the insulin/glucagon ratio was lower in preterm infants ($p<0\ 01$)

There was no relation between the glycemic responses to L alanine and the basal levels of D-BOHB (Fig 3)

Effect of glucagon administration

In six preterm infants plasma glucose rose from 3 35±0 1 to 6 05±0 27 mmol/l thirty

Table 3 *Effect of intravenous administration of L alanine (150 mg/kg) on plasma insulin glucagon concentrations and insulin/glucagon molar ratio*

		Time (min)				
	No	0	10	?0	30	40
Glucagon (pmol/l)						
Term infants	10	53±5	70±8	65±6	67±7 *	61±6
Preterm infants	11	61±6	70±10	75±9	75±9	76±7
Insulin (mU/l)						
Term infants	10	12±1	15±?	14±1	14±1	13±1
Preterm infants	11	12±1	17±3	15±2	13±2	11±1
Insulin Glucagon (molar ratio)						
Term infants	10	1 79±0 22	1 61±2 8?	1 49±0 15	1 58±0 ?6	1 71±0 25
Preterm infants	11	1 54±0 ?4	2 11±0 56	1 43±0 ?1	1 3?±0 16	1 26±0 14

Mean ±S E M
Comparison with baseline level= $p<0\ 05$ (*t* test) $p<0\ 01$ (Wilcoxon s test) Comparison between groups= $p<0\ 01$

Table 1 *Summary of clinical data*

Subjects	No	Birthweight g		Gestational age weeks		Postnatal age hours		Complications	
		Mean	Range	Mean	Range	Mean	Range		
Term	10	3250	(2880–3700)	40 13	(38–42)	50 8	(30–76)	Cesarean sections	2
Preterm	11	1919	(1800–2570)	35	(31–36)	47 4	(29–72)	Cesarean sections	?
								Toxemia	1
								Twins	1

D BOHB pyruvate lactate insulin and glucagon in appropriate term and preterm newborn infants

MATERIAL AND METHODS

All the newborn infants were investigated during the first three days after birth The study procedures were approved by the ethic committee of the Department of Neonatology and of the Unité de Recherche U 34 All the infants were appropriate for gestational age their birth weight being between the 10th and the 90th percentile of the intrauterine growth curve (15) The gestational age was determined by the menstrual history and by clinical assessment according to the method of Dubowitz & al (5) (Table 1) Infants with Apgar score lower than six and infants whose mother previously received beta methasone injections were excluded No infants developed hypoglycemia (i e blood glucose lower than 1 65 mmol/l)

Feeding was started at the age of six hours The infants were fed with a commercial formula or with maternal milk at intervals of three or four hours A 10% glucose infusion was administrated to provide for a total daily input of 8 g of glucose per kg Feeding and infusion of glucose were discontinued three hours before the test and an isotonic saline solution was infused during the test at the rate of 0 05 ml/min

A sterile solution of L alanine prepared in the laboratory of the hospital was injected at the dose of 150 mg/kg of body weight into a scalp vein over a period of one minute Blood samples were collected before and 10 20 30 and 40 min after L alanine infusion through an arterial umbilical catheter previously inserted only blood glucose pyruvate and lactate were studied at 50 min to reduce the amount of blood drawn At the end of the test glucagon (300 µg/kg) was injected into a peripheral vein in six premature infants and plasma glucose was assessed before and 30 min after

0 1 ml of blood was collected for glucose assay immediately centrifuged and plasma glucose was analysed by a glucose oxydase method (Beckman glucose analyser) Specimens of 0 5 ml collected in heparinized microtubes were immediately deproteinized by perchloric acid precipitation (0 6 M) and blood pyruvate and lactate were determined by an enzymatic method (27) Blood samples were collected in heparinized microtubes for insulin (0 5 ml) and in tubes prepared with EDTA and trasylol for glucagon determinations (1 5 ml) The tubes were kept in ice and immediately centrifuged at 4 C plasma was removed and frozen until assayed The samples were analysed for insulin by the method of Hales & Randle (17) and for glucagon by a radioimmunoassay which did not cross react with purified gut glucagon (21) D-BOHB was measured by an enzymatic method (29) The statistical analysis were performed by means of Wilcoxon s test and paired *t* test

RESULTS

Effects of alanine

In *term infants* intravenous L alanine resulted in a significant increment of plasma glucose over baseline level within 30 40 50 min ($p<0.01$) (Fig 1) It produced a significant rise in plasma glucagon in 10 to 40 min ($p<0.01$) but

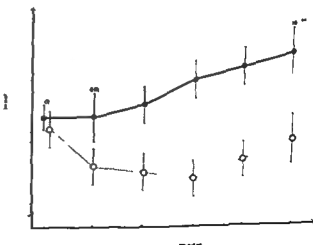


Fig 1 Effect of L alanine on plasma glucose concentrations (mean ± S E M) in preterm ( ) and term (—) infants Comparison with baseline level = *$p<0.05$ *$p<0.01$ Comparison between groups = +$p<0.05$ ++$p<0.01$

Table 2 *Effect of intravenous administration of L alanine (150 mg/kg) on blood pyruvate and lactate concentrations*

	No	Time (min) 0	10	20	30	40	50
Pyruvate (mM/l)							
Term infants	5	0 10±0 03	0 09±0 02	0 10±0 03	0 09±0 03	0 05±0 01	[illegible] 14±0 07
Preterm infants	8	0 09±0 01	0 06±0 01	0 07±0 01	0 09±0 01	0 09±0 02	0 09±0 02
Lactate (mM/l)							
Term infants	[illegible]	2 76±0 8	2 46±0 8	2 56±0 9	2 90±1 1	2 91±1 1	[illegible] 32±0 8
Preterm infants	8	2 36±0 2	2 05±0 1	2 61±0 4	2 95±0 6	3 01±0 5	3 22±0 5

no significant change in plasma insulin (Table 3) There was no significant change of insulin/glucagon molar ratio and of blood pyruvate and lactate levels (Table 2) The blood concentration of D BOHB decreased after 10 min ($p<0$ 05) and remained under the baseline level ($p<0$ 01) (Fig 2)

In *premature infants* plasma glucose decreased after alanine administration ($p<0$ 05) and returned to the baseline level (Fig 1) The plasma glucagon concentration rose significantly ($p<0$ 05) but no change of plasma insulin was detected (Table 3) Insulin/glucagon molar ratio remained similar to the base line value There was no changes of blood pyruvate lactate (Table 2) and D-BOHB levels ($p>0$ 05) (Fig 2)

Comparison between the two groups The basal levels of plasma glucose insulin and glucagon blood pyruvate and lactate were similar in both groups ($p>0$ 05) The baseline levels of D-BOHB were however lower in preterm than in term infants ($p<0$ 05) After alanine administration plasma glucose and blood D-BOHB were lower in preterm infants There was no difference for the lactate and pyruvate levels the insulin and glucagon concentrations After 40 min the insulin/glucagon ratio was lower in preterm infants ($p<0$ 01)

There was no relation between the glycemic responses to L alanine and the basal levels of D-BOHB (Fig 3)

Effect of glucagon administration

In six preterm infants plasma glucose rose from 3 35±0 1 to 6 05±0 27 mmol/l thirty

Table 3 *Effect of intravenous administration of L alanine (150 mg/kg) on plasma insulin glucagon concentrations and insulin/glucagon molar ratio*

	No	Time (min) 0	10	20	30	40
Glucagon (pmol/l)						
Term infants	10	53±5	70±8	65±6	67±7	61±6
Preterm infants	11	61±6	70±10	75±9	75±9	76±7
Insulin (mU/l)						
Term infants	10	1?±1	15±?	14±1	14±1	13±1
Preterm infants	11	12±1	17±3	15±2	13±2	11±1
Insulin/Glucagon (molar ratio)						
Term infants	10	1 79±0 ??	1 61±? 8?	1 49±0 15	1 58±0 26	1 71±0 25
Preterm infants	11	1 54±0 24	2 11±0 56	1 43±0 21	1 37±0 16	1 26±0 14

Mean ±S E M
Comparison with baseline level= $p<0$ 05 $p<0$ 01 (Wilcoxon s test) Comparison between groups= $p<0$ 01 (t test)

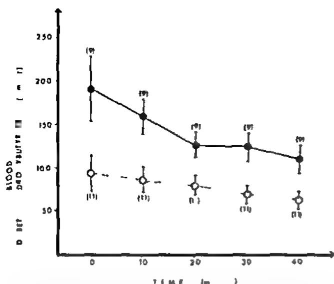


Fig 2 Effect of L alanine on blood betahydroxybutyrate concentrations (mean ±S E M) in term (——) and preterm ( ) infants Comparison with baseline level=*$p<0\ 05$ **$p<0\ 01$ Comparison between groups=+$p<0\ 05$ ++ $p<0\ 01$

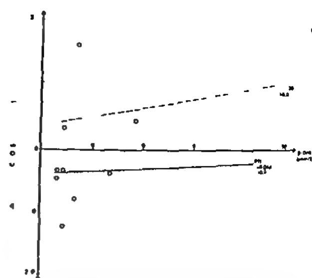


Fig 3 Relationship between basal betahydroxybutyrate (BOHB) concentration and glycemic response (Δ glucose) in full term (● FTI) and preterm (O PTI —) infants

minutes after glucagon administration ($p<0\ 01$)

DISCUSSION

The effects of oral and intravenous alanine on blood glucose concentrations have been studied in full term newborn infants and/or in newborn infants who were appropriate for their gestational age (4 9, 16 30 31) But there is no report comparing this effect in full term and premature infants This study shows a different glycemic response to L alanine infusion in term and preterm newborn infants at similar postnatal ages

These two types of glycemic response to L alanine infusion are not explained by different hormonal secretion Plasma insulin concentrations did not change significantly in either group Glucagon secretion was stimulated by L alanine infusion in both groups These results agree with previous findings showing that the endocrine activity of pancreas is adequate for the metabolic state in neonatal life in term (25 31) and preterm (6) infants

Despite similar hormonal response to i v L alanine administration only term infants were able to increase their hepatic glucose output in response to increased circulating levels of glucagon Although the increment in glucose seems small in comparison to the glucagon response to L alanine (25) it agrees with experimental studies showing that glucagon participates significantly in perinatal glucose homeostasis (26) These results are different from the findings of Williams et al (30) who observed no change in plasma glucose after L alanine administration in infants appropriate for their gestational age older than 24 hours There were however term and preterm infants in their control group Our results in term infants agree with the findings of Wise et al (31) They are different from those of Lowry & Adam (14) who observed in term infants 30 min after birth no effect of alanine infusion on plasma glucose (or lactate and pyruvate) concentrations despite the fact that mean plasma glucagon concentration rose significantly from 15 to 40 pmol/l These results suggest that glucagon activity depends on the postnatal age in term infants

In preterm infants the rise in plasma glucagon levels could only keep the concentra

tion of plasma glucose at its baseline level despite a lower insulin/glucagon ratio after 40 min This rise in glucagon was apparently inadequate to evoke glycogenolytic and/or gluconeogenic responses in order to raise plasma glucose concentrations since only glucagon in pharmacologic doses was capable of increasing glucose concentrations Low glycogen stores have been reported in preterm infants (24) but hardly explain the poor response to circulating glucagon since these infants were given large amounts of glucose The disposal of glucose in preterm infants resembles that of term newborn infants (1 3) Preterm infants had significantly lower basal concentration of D-β hydroxybutyrate when compared to term infants however there was no relationship between the basal D-β hydroxybutyrate levels and the glycemic responses to L alanine Therefore these results suggest a lower sensitivity to physiological concentrations of glucagon in preterm infants This reduced sensitivity could explain the initial decrease in plasma glucose after L alanine administration (18) It could be similar to the role of ontogenesis in hepatic responsiveness to glucagon described in newborn rats (28)

Alanine reverses some of the metabolic consequences of diminished gluconeogenesis and leads in adults to a prompt decrease in the blood level of β hydroxybutyrate (11) β hydroxybutyrate is a main alternative fuel to glucose in newborn infants (2 23) and the relationship between gluconeogenesis and ketogenesis plays an important role in perinatal metabolism (8 10 13) However the effect of L alanine on D-BOHB levels has not been investigated in human newborn infants The anti ketogenic effect of L alanine infusion was confirmed in full term infants It was not observed in preterm infants probably because of lower levels of D-BOHB In previous reports blood D-BOHB concentrations were similar in term and preterm infants (10 22) The discrepancy of this study could be explained by the conditions of the investigation and probably by glucose infusion (22)

The mechanism of the antiketogenic effect of alanine is not fully understood In newborn infants it could not be explained by changes in plasma insulin glucagon blood pyruvate and lactate concentrations This is in agreement with similar findings in human adults (11) and with experimental studies in rats (19) Investigations performed recently in rats suggest that this antiketogenic effect is not due to a modification of peripheral utilization of D BOHB but depends upon the redox state of liver mitochondria (20) Further investigations are required to know whether or not this finding could be applied to human newborn infants

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COPPER DEFICIENCY AND HYPOCALCEMIC RICKETS IN A SMALL FOR DATE INFANT

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ABSTRACT Sann L David L Galy G and Romand Monier M (Service de Neonatologie du Professeur Maurice Bethenod and Inserm U 34 Hopital Debrousse and Laboratoire de Medecine Nucleaire Hopital Neurologique Lyon France) Copper deficiency and hypocalcemic rickets in a small for-date infant Acta Paediatr Scand 67 303 1978.—A case of copper deficiency associated with hypocalcemia radiological features of rickets and hyperparathyroidism is described in a small for-date infant (gestational age 39 weeks B W 1 240 g) Neonatal serum copper (Cu) levels were found between 22 3 and 13 8 μmol/l She was given daily 2 400 IU of vitamin D and a load dose of 80 000 IU at the age of 55 days At the age of 79 days X rays of the legs and wrist showed spread cupped and frayed metaphyses Serum Ca was 1.35 mmol/l P=0 99 mmol/l with high alkaline phosphatases (A P) 590 IU/ml But plasma level of 25 hydroxycholecalciferol (25 OH CC) was normal=10 8 ng/ml Serum Cu was low=3 [illegible] μmol/l and serum immunoreactive parathormone (iPTH) level was elevated 5 0 μlEq/ml (N⩽100) Administration of vitamin D (15 mg) induced an immediate normalization of serum Ca normal serum iPTH (68 μlEq/ml) in one month normal X rays in two months and normal A P in four months Serum Cu and ceruloplasmin levels increased slowly without any supplementation to subnormal levels at the age of eight months (14 9 and 1 65 μmol/l Serum Cu concentrations were found to be normal (16 0–33 7 μmol/l) in five children with hypocalcemic rickets These results suggest a role of Cu deficiency in the occurrence of this transient vitamin D resistant rickets

KEY WORDS Rickets hypocalcemia copper depletion small for-date infant

Although the low birthweight infant is at risk from copper deficiency by three months after birth (9) there are few reports of this syndrome (1 3) We present a new case which was associated to hypocalcemic rickets despite normal vitamin D administration

CASE HISTORY

Marie Thérèse is the third child of a 30-year-old caucasian woman whose height is 1 40 m and weight 52 kg Her first child a 7 year old boy is in good health Her second child was a girl who died at the age of three days from hydrocephalus This third pregnancy was uneventful until the 6th week when low urinary oestriol was found A cesarean section was performed at 39 weeks because of fetal distress Placenta weight was 170 g Apgar score was 8/10 The B W 1 240 g length 38 5 cm and head circumference [illegible] cm were all below the tenth percentile of Lubchenco s growth curve (11) Physical examination was satisfactory Initial laboratory tests revealed transient hypoglycemia (0 5 mmol/l) IgM concentration was 10 mg/100 ml There were 5 100 000 erythrocytes per mm³ with 199 g/l hemoglobin 27 500 white cells with 50% neutrophils and 40 000 platelets per mm³ Serum electrolytes concentrations were normal At the second day serum calcium (Ca) dropped to 1 62 mmol/l but was restored with intravenous Ca supplementation 25 hydroxy colecalciferol (15 μg) and parathormone Lilly (50 USP) administration The karyotype was XX Subsequent evolution was subnormal The infant was fed with a commercial infant formula providing 67 calories/100 ml calcium 1 42 mmol/100 ml phosphorus 1 40 mmol/100 ml copper 31 4 μmol/100 ml and zinc 178 μmol/100 ml Feeding was started at the first day with 60 ml/kg and it reached 200 ml/kg/24 hr at the age of 18 days In addition 12 000 IU of vitamin A 50 mg of vitamin C and 2 400 IU of vitamin D_2 were given daily from 15 to 51 days of age The lowest erythrocyte count was observed at the age of 32 days with 2 950 000 per mm and [illegible] g/l hemoglobin but no blood transfusion was performed At the age of 38 days X rays of the legs showed mild osteoporosis The child was discharged from the hospital after administration of 80 000 units of vitamin D at the age of 55 days (weight 2 400 g)

She was referred to Hôpital Debrousse 24 days later for bronchitis and failure to thrive Her weight was 3 100 g length 48 cm and head circumference 36 5 cm After an initial fever (38°8C) a tendency towards hypothermia (35°4



Fig 1 Roentgenogram of the legs and the wrist at the age of 79 days The metaphyses are spread cupped and poorly mineralized The diaphyses show subperiosteal new bone

to 36 2) was noticed Physical examination showed a pale skin with mild eczematic dermatitis behind the ears on the neck and shoulders A grade 2 soft systolic murmur was heard but no organ enlargement was found Skull bones were markedly soft the ribs seemed enlarged and mild wrists swelling were found Hemoglobin was 90 g/l with 30% hematocrite There were 7700 white cells per mm³ with 20% neutrophils 63% lymphocytes and 9% monocytes Roentgenogram of the chest showed mildly enlarged anterior ribs and X rays of the legs and wrist revealed metaphyseal cupping and flaring with poor mineralization mild osteoporosis with subperiosteal new

Table 1 *Evolution of the biochemical and radiological data—serum calcium (Ca) phosphorus (P) immunoreactiv[illegible] parathyroid hormone (iPTH) and plasma 25 hydroxycholecalciferol (25 OH CC) levels*

X ray aspects N=normal R=rickets O=osteoporosis

Age									
Days	1	2	15	25	38	55	79	80	82
Months				1		2			
Therapy									
25 OH CC (μg)		15							
Vit D_2 (IU)			←2 400/day→			↓ 80 000	(Ca infusion)		
Serum copper (N=17 3±3 1 μmol/l)			22 3	13 8		23 1	3 1		
Serum ceruleoplasmine (N=2 06±0 3 μmol/l)									
Serum Ca (mmol/l)	2 25	1 62	2 35			2 42	1 35	1 75	1 7[illegible]
Serum Mg (mmol/l)		0 73					0 81		0 77
Serum [illegible] (mmol/l)	1 47		1 50			1 82	0 99		0 99
Alkaline phosphatase (N≈300 IU/ml)	130		120			460	590		720
Serum iPTH (N≤100 μlEq/ml)									520
Plasma 25 OH CC (N=13 2±4 4 ng/ml)									10 8
X rays (legs)			N	O		O	R		[illegible]

One lo a dose

bone along the diaphyses (Fig 1) Hypocalcemia (1 35 mmol/l) was detected without any clinical manifestation Serum magnesium concentration was normal (0 81 mmol/l) and serum copper level low (3 14 μmol/l) After intravenous administration of calcium (1 12 mmol/kg/6 hours as calcium gluconate) serum Ca rose to 2 17 mmol/l Despite I V Ca supplementation (1 12 mmol/kg/24 hr) it dropped to 1 72 mmol/l and a large dose of vitamin D_2 (15 mg) was given I M to obtain normal and stable serum Ca concentration (Table 1)

The girl was again referred to Hopital Debrousse at the age of 4½ months for bronchitis Physical examination showed a pale girl weighing 3 700 g and 52 cm in height Except for slight psychomotor retardation with hypotonia the child behaved and reacted normally The same systolic murmur was heard Craniotabes and swelling of the bone s end had disappeared Erythrocytes count was 3 450 000 per mm² with 9 2/100 ml hemoglobin 8 600 white cells per mm² and [illegible] % neutrophils Serum Ca and P levels were normal but alkaline phosphatases elevated (744 IU/ml) (Table 1) Serum copper level was still low (3 4? μmol/l) X rays of the legs showed regular metaphyses better mineralized but still cupped and enlarged The baby was discharged without treatment

At the age of eight months she was an active girl looking well but with subnormal psychomotor development Laboratory tests and X rays of the bones were normal

MATERIALS AND METHODS

Serum Ca was determined by atomic absorption (spectrophotometer IL Inc 353) serum P according to Fiske & Subbarow (7) and serum alkaline phosphatase by

84	[illegible]	87	138		
		4		5	8
↓ 600 000					
			3 4	6 4	14 9
			0 40	0 73	1 65
	2 35	2 45	2 57	2 45	2 45
	1 03	0 80			
	1 37	1 79	1 76	2 17	1 98
	764	750	744	5 0	80
		370	68	96	
		8 5			
		R	±	N	N

the method of Bessey et al (5) Serum Cu was measured by the method of Parker et al (17) normal values in adults are 17 3±3 1 μmol/l Serum ceruloplasmin was determined according to the method of Bizollon & Galy (6) normal values in adults are [illegible] 6±0 3 mmol/l

Serum immunoreactive parathyroid hormone (iPTH) was determined by radioimmunoassay using an antibovine parathyroid hormone guinea pig antiserum (GP6) a gift of Dr Constantine Anast from the Department of Pediatrics University of Missouri Hyperparathyroid serum was used as a standard reference Normal range in children and adults never exceeded 100 microliters equivalents per milliliter (μlEq/l) Approximately 90% of normal subjects had detectable levels of serum iPTH (4) The lower limit of detectability of the assay was 10 μlEq/ml Plasma 25 hydroxycholecalciferol (25-OH-CC) was measured by a competitive proteinbinding method modified from the assay of Preece et al (13) Normal values in infants are 13 2±4 4 ng/ml

RESULTS

The mother s serum Cu (15 [illegible] μmol/l) and ceruloplasmin (1 65 μmol/l) concentrations were found to be subnormal

The data from the infant are shown in Table 1 Serum Cu levels detected during the neonatal period were at the upper limit of normal range A very low Cu concentration was found at the age of 79 days when the infant developed hypocalcemia and at the age of 138 days with low level of ceruloplasmin Subsequently serum levels of Cu and ceruloplasmin rose slowly and spontaneous to subnormal values At the age of 79 days when hypocupremia and hypocalcemia were detected the infant developed hypophosphoremia (0 99 mmol/l) with high level of serum iPTH (520 μlEq/ml) but normal 25 OH CC concentration (10 8 ng/ml) Five days after Ca supplementation and vitamin D_2 administration serum Ca and P levels were normal but serum iPTH concentration was still elevated Normal serum iPTH concentration was observed at the age of four months and of serum alkaline phosphatase at the age of eight months

Serum Cu concentrations were controlled at the age of three months in eight term small for-date infants All were fed with the same milk The mean was 14 6 μmol/l (range 8 95–18 88) Serum Cu concentrations were



Fig 1 Roentgenogram of the legs and the wrist at the age of 79 days The metaphyses are spread cupped and poorly mineralized The diaphyses show subperiosteal new bone

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Serum ceruleoplasmine (N=2 06±0 3 μmol/l)									
Serum Ca (mmol/l)	2 25	1 62	2 35			2 42	1 35	1 75	1 72
Serum Mg (mmol/l)		0 73					0 81		0 77
Serum P (mmol/l)	1 47		1 50			1 82	0 99		0 99
Alkaline phosphatase (N=300 IU/ml)	130		120			460	590		720
Serum iPTH ($N \leq$100 μlEq/ml)									520
Plasma 25 OH CC (N=13 2±4 4 ng/ml)									10 8
X rays (legs)			N	O		O	R		R

One lo ▪ dose

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also measured in five infants (age 3 to 5 months) with typical hypocalcemic rickets due to dietary deficiency of vitamin D The mean was 26 0 μmol/l (range 16 0–33 7 μmol/l)

DISCUSSION

In this infant copper deficiency and transient vitamin D resistant rickets were associated

Pure copper deficiency syndrome is very rare and we are aware of only two previous reports in premature infants (1 3) In addition it has been also suspected in other premature infants by Griscom et al (8) The clinical features included failure to thrive apathy hypotonia seborrheic dermatitis anemia neutropenia and osteoporosis (1 3 8) In this child copper deficiency was demonstrated by two measurements of serum copper concentration The low serum ceruleoplasmine detected at the age of four months can be secondary to copper deficiency (10) Neverthe less the clinical manifestations were incomplete and there was no neutropenia

The origin of this copper deficiency is not known Three factors could be involved in this case 1) Low copper body stores may contribute to the risk from Cu deficiency in premature infants (9 10) but copper deficiency has never been reported in term small for date infants In eight controls serum Cu concentrations were found to be normal a finding suggesting that fetal malnutrition does not seem to induce deficiency In this case maternal and neonatal serum Cu concentrations were normal excluding depleted body stores of fetal origin 2) Low dietary Cu intake There is general agreement that the daily intake of copper should be at least 50 μg/kg (9) and even 90μg/kg in premature infants (14) It is clear that this child was not given these amounts and the later increase in serum Cu concentrations could probably be explained by introduction of semisolid and solid infant food However Wilson & Lahey (18) failed to induce dietary copper deficiency and hypocupremia in premature infants at 60 days of age In addition the eight other small for date infants fed in a similar way had no hypocupremia 3) Decreased intestinal copper absorption cannot be excluded since it was not studied in this patient Molybden excess (15) or abnormal Cu/Zn dietary ratio (17) can account for abnormal Cu absorption However these explanations are unlikely since eight control infants fed with the same milk did not develop copper deficiency at the same age

The association of skeletal changes (cupping and flaring of long bone s metaphyses) and biological features (hypocalcemia hypophosphoremia elevated alkaline phosphatases and hyperparathyroidism) is highly suggestive of rickets The diagnosis was confirmed by healing of the bone lesions and by normalization of serum abnormalities by vitamin D_2 administration Plasma 25 OH CC levels are low in infantile rickets especially at a stage with major metaphyseal changes (2) but this biological parameter of rickets was normal in this patient In addition she had previously been given large doses of vitamin D The reason for this discrepancy is not clear and its association with copper deficiency must be pointed out It is unlikely that copper deficiency is secondary to rickets since serum Cu concentrations were found to be normal in five infants with hypocalcemic rickets Therefore copper could play a role for vitamin D metabolism However the role of copper deficiency was not demonstrated in this infant since no copper supplementation was given In addition hypocalcemia and hypophosphoremia have never been reported in copper deficiency and the histological findings in the bone lesions seem to be different from rickets (15) Finally a direct effect of copper on the bones is also possible

In conclusion a case is presented associating copper deficiency with hypocalcemic rickets despite normal 25 OH CC level and vitamin D administration This association raises the question of a possible role of copper deficiency on the occurrence of transient vitamin D resistant rickets

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DIAGNOSTIC VALUE OF HAND X RAYS IN TURNER S SYNDROME

S NECIĆ and D B GRANT

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ABSTRACT Necić S and Grant [redacted] B (Paediatric University Clinic Belgrade Yugoslavia and the Hospital for Sick Children Great Ormond Street London England) Diagnostic value of hand X rays in Turner s syndrome Acta Paediatr Scand [redacted] 309 1978.—Previously described radiological signs of Turner s syndrome were evaluated in X rays of the left hand from 17 patients with Turner s syndrome (age 10 4–15 3 years) and 17 age-matched girls with constitutional short stature While none of the signs clearly distinguished between the two groups ballooning of the tips of the terminal phalanges with a high ratio between the tip and mid-shaft diameters seemed [redacted] be the most useful sign of Turner s syndrome Disproportionately long phalanges in the 4th finger and the presence of a coarse reticular pattern in the carpal bones appeared [redacted] be the next most useful signs The presence of a short 4th metacarpal or a narrow carpal angle and assessment for Madelung s deformity were of little value in distinguishing between the two groups

KEY WORDS Turner s syndrome hand X rays metacarpals phalanges osteoporosis

A number of minor radiological abnormalities have been described in the hands of patients with Turner s syndrome These include a short 4th metacarpal (1) a narrow carpal angle (6) Madelung s deformity (8) relatively long phalanges with tufting of the tip of the terminal phalanx (7) and a coarse reticular pattern in the carpal bones (2) Most of the published studies have been based on patients of widely differing ages This paper compares the incidence of these anomalies in 17 young girls with Turner s syndrome and 17 age matched girls with constitutional short stature

PATIENTS AND METHODS

Patients and controls

X rays of the left hand and wrist taken between the ages of 10 4 years and 15 3 years (mean 13 0 years) in 17 patients with Turner s syndrome were examined Ten of these patients showed an XO karyotype and the remainder had an XX/XO or Xx/XO pattern

X rays from 17 girls attending the growth clinic with constitutional short stature were used [redacted] controls

Assessment of X rays

One film from each patient with Turner s syndrome and a corresponding X ray from the control group were matched for age to within 2 months All the X rays were assessed by two paediatricians for (*a*) the presence of a coarse reticular pattern in the carpal bones (²) (*b*) tufting [redacted] ballooning of the tips of the terminal phalanges (7) and (*c*) abnormal tilting of the lower end of the radius with projection beyond the lower end of the ulna (Madelung s deformity) (Fig. 1) Each observer examined the coded X rays in random order on 2 occasions and films considered abnormal on one or both occasions were scored as positive

In addition the metacarpal sign (4) the ratio between the lengths of the 4th and 5th metacarpals (1) the difference between the combined lengths of the proximal and distal phalanges of the 4th finger and the length [redacted] the 4th metacarpal (7) the ratio between the tip and mid shaft diameters of the terminal phalanx of the 4th finger (7) the carpal angle (6) and the cortex/length ratio $((D-d^2)/D\times L)$ of the second metacarpal were measured in each film by one observer The last of these ratios has been described as an index of osteoporosis by Gryfe et al (5)

The significance of differences between mean values in the 2 groups [redacted] patients was tested with Student s *t* test

RESULTS

The results are summarized in Table I

a) Carpal reticular pattern Both observers scored this as abnormal in 11 of the girls with Turner s syndrome and 2 controls and normal

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Table 1 *Positive radiological findings in girls with Turner's syndrome and constitutional short stature*

Radiological sign	Turner's syndrome (17)	Constitutional short stature (17)
a) Coarse carpal reticular* pattern	11	2
b) Tufting of terminal* phalanges	15	1
c) Madelung's deformity*	7	3
d) 4th distal phalanx tip/midshaft diameter >1 46	15	1
e) 4th finger distal phalanx + proximal phalanx − metacarpal >4 mm	10	2
f) 4th/5th metacarpal ratio <1 03	6	0
g) Metacarpal sign	8	3
h) Carpal angle <117	3	1

* Scored positive by both observers

tal phalanges were at least 4 mm greater than the length of the corresponding 4th metacarpal The mean differences in length for the Turner group (3 8 mm) and the controls (1 0 mm) were significantly different from one another ($t=3\ 75$ $p<0\ 001$)

f) *Metacarpal sign* This was scored as positive in 8 girls with Turner's syndrome and 3 controls

g) *4th/5th metacarpal ratio* This ratio was below 1 03 in 6 of the Turner patients and in none of the controls However the difference between the mean values for the two groups (Turner 1 05 Control 1 08) was barely significant ($t=2\ 75$ $p<0\ 05$)

h) *Carpal angle* Three Turner patients and one control had carpal angles of less than 117° There was no obvious difference in the angle between the 2 groups and the average values were almost identical (Turner 124 7 control 125 1°)

i) *2nd metacarpal cortex/length ratio* This ratio was not significantly different in the Turner patients (mean 0 088) and the controls (mean 0 092)

DISCUSSION

The above results illustrate many of the minor radiological abnormalities which have been described in Turner's syndrome While previously published results clearly indicate that none of these signs is invariably present in Turner's syndrome most of the series are based on patients of widely differing ages Our results indicate that even in a relatively narrow age range radiology of the hand has a limited place in recognition of the disorder

The relatively high incidence of discrepancies between the two observers illustrates the problem of defining anomalies such as a coarse reticular pattern in the carpal bones phalangeal tufting or Madelung's deformity which are difficult to quantitate However despite this limitation the first two of these signs are probably useful in many cases

Objective results obtained by measurement of bone length or diameter might be expected to give more reliable diagnostic information In this small series the ratio between the tip and mid shaft diameters of the distal phalanx of the fourth finger seemed to differentiate most clearly between the Turner and control patients This ratio which provides a fairly objective index of tufting was greater than 1 46 in all but 2 of the Turner patients but in only one of the controls This expansion of the terminal phalanges in Turner's syndrome may be related to a generalized over growth of the finger tips in fetal life which also leads to a high dermal ridge count (9)

A short 4th metacarpal is one of the text book signs of Turner's syndrome but was not particularly impressive in this study either in terms of the metacarpal sign or the ratio between the lengths of the 4th and 5th metacarpals Disproportion between the phalangeal and metacarpal lengths in the 4th finger appears to be a more useful sign A narrow carpal angle appears to have very little diagnostic value in this age group

The abnormal carpal reticular pattern in Turner's syndrome has been attributed to osteoporosis (2) In this study the cortex/

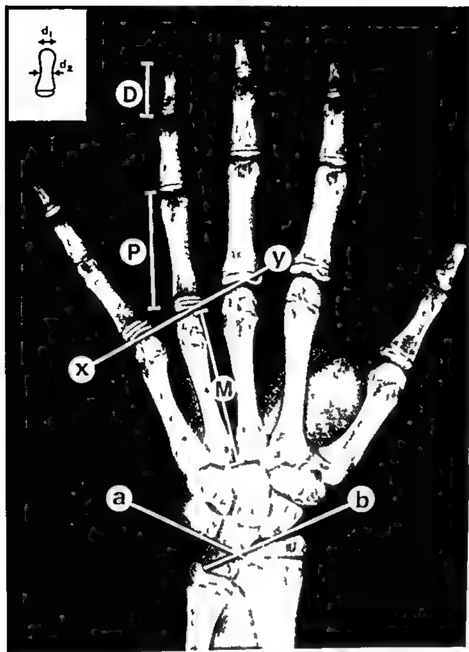


Fig 1 Hand X ray in a 13 9 year old girl with Turner s syndrome showing (*a*) coarse carpal reticular pattern (*b*) tufting of the terminal phalanges (*c*) Madelung s deformity (*d*) Disproportionately long phalanges (D+P−M=3 mm) (*e*) Phalangeal tip/midshaft ratio of 1 61 (insert) (*f*) Positive metacarpal sign (x−y) (*g*) normal carpal angle (130°) (*a*–*b*)

in 3 Turner patients and 11 controls Discordant results were obtained in 7 subjects (3 Turner 4 control)

b) *Tufting of the terminal phalanges* Both observers considered this sign positive in 15 Turner patients and one control and negative in 10 controls Observations were discordant in 8 subjects (2 Turner 6 controls)

c) *Madelung s deformity* Both observers agreed that 7 Turner patients and 3 controls had Madelung s deformity while 5 Turners and 10 controls did not have this sign Discordant observations were made in 9 patients (5 Turner 4 control)

d) *Tip/mid shaft diameters of the 4th distal phalanx* This ratio was greater than 1 46 in 15 of the Turner patients and in one control patient The mean value for the Turner group (1 58) was significantly greater than the mean value for the controls (1 34) (t=6 83 p<0 001)

e) *Phalangeal and metacarpal lengths (4th finger)* In 10 Turner patients and 2 controls the combined lengths of the proximal and dis

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) Madelung s deformity[a]	7	3
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) 4th finger distal phalanx + proximal phalanx metacarpal >4 mm	III	2
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h) Carpal angle <117°	3	1

Scored positive by both observers

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h) *Carpal angle* Three Turner patients and one control had carpal angles of less than 117° There was no obvious difference in the angle between the 2 groups and the average values were almost identical (Turner 124 7 control 125 1)

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The relatively high incidence of discrepancies between the two observers illustrates the problem of defining anomalies such as a coarse reticular pattern in the carpal bones phalangeal tufting or Madelung s deformity which are difficult to quantitate However despite this limitation the first two of these signs are probably useful in many cases

Objective results obtained by measurement of bone length or diameter might be expected to give more reliable diagnostic information In this small series the ratio between the tip and mid shaft diameters of the distal phalanx of the fourth finger seemed to differentiate most clearly between the Turner and control patients This ratio which provides a fairly objective index of tufting was greater than 1 46 in all but 2 of the Turner patients but in only one of the controls This expansion of the terminal phalanges in Turner s syndrome may be related to a generalized over growth of the finger tips in fetal life which also leads to a high dermal ridge count (9)

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The abnormal carpal reticular pattern in Turner s syndrome has been attributed to osteoporosis (2) In this study the cortex/

length ratio in the 2nd metacarpal was estimated as this ratio has been described as a useful index of osteoporosis in adults and children (5) As judged by this ratio our patients with Turner s syndrome did not have osteoporosis but it was often difficult to define the inner margin of the cortex particularly in the Turner patients and as a result the ratio may have been overestimated in some cases While Brown et al (3) found no signs of cortical thinning in 8 young girls with Turner s syndrome 6 of their subjects showed evidence of decreased bone density and increased bone resorption and these features probably provide more reliable indices of early osteoporosis in young patients

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RENAL FUNCTION IN IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

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ABSTRACT Broberger U & Aperia A (Departments of Paediatrics at Karolinska Sjukhuset and St Goran s Children s Hospital Stockholm Sweden) Renal function in idiopathic respiratory distress syndrome Acta Paediatr Scand 67 313 1978.—Renal function was studied in 11 pre term infants with idiopathic respiratory distress syndrome (IRDS) grade 1 according to Prod hom s criteria As a reference 16 healthy pre-term infants were studied The groups did not differ with regard to mean gestational age (GA) and mean post natal age (PNA) The studies were preformed twice first at a PNA of 33–37 hours and then at 132–148 hours GFR and C_p were determined with the single injection technique and the ability to excrete Na was determined following an oral Na load GFR was higher in IRDS infants at the first investigation and slightly lower in IRDS infants at the second investigation The GFR correlated to the lowest recorded P_{aO_2} ($r=0.45$) in IRDS infants C_{PAH} was similar in IRDS and controls at the first and lower in IRDS infants at the second investigation The urinary Na excretion was significantly higher in IRDS infants Treatment with digitalis was in part responsible for the high urinary Na excretion The IRDS infants had a higher Na and glucose intake than the control infants It is suggested that this higher intake is in part responsible for the relatively high GFR and urinary Na excretion in the IRDS infants

KEY WORDS GFR C_{PAH} urinary sodium excretion neonate idiopathic respiratory distress syndrome

This study describes renal functional changes during the course of clinically well-defined IRDS It is an old observation that impairment of renal function occurs in IRDS (9) More recent systematic studies however have failed to reveal the factors in IRDS infants that predispose to impaired renal function Guignard et al (14) found a marked decrease in the GFR in all infants studied On the other hand Siegel et al (22) found no effect of IRDS on renal function in the infants they studied The differences in result may depend on differences in severity of IRDS as well as on the fact that there are usually several uncontrolled variables involved in physiological studies of newborn infants Renal function develops rapidly during the neonatal period and depends on gestational and postnatal age (16 18) Fluid intake and hematocrit may also influence renal function as may the pharmacological treatment of the patient In this study great care was taker to control these factors

One of the important consequences of disturbed renal function is a reduced ability to control salt and fluid balance It is well established that the ability to excrete sodium is low in healthy newborn infants (3) Infants with IRDS need correction of acidosis Sodium bicarbonate is commonly used for this purpose Determinations of renal Na^+ tolerance in IRDS infants was therefore included in this study

MATERIALS

Eleven pre term infants with idiopathic respiratory distress syndrome (IRDS) and sixteen healthy pre term infants were studied during the second and sixth days of

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length ratio in the 2nd metacarpal was estimated as this ratio has been described as a useful index of osteoporosis in adults and children (5) As judged by this ratio our patients with Turner s syndrome did not have osteoporosis but it was often difficult to define the inner margin of the cortex particularly in the Turner patients and as a result the ratio may have been overestimated in some cases While Brown et al (3) found no signs of cortical thinning in 8 young girls with Turner s syndrome, 6 of their subjects showed evidence of decreased bone density and increased bone resorption and these features probably provide more reliable indices of early osteoporosis in young patients

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'able 2 *Oxygen concentration in inspired air (FiO₂) and laboratory data in 11 infants with IRDS t the 1st (age 37 hrs) and 2nd (age 148 hrs) investigation*

	FiO_2 %		P_{aO_2} (mmHg)			pH		P_{aCO_2} (mmHg)		Hematocrit (%)		Serum Na conc (mmol/l)	
	I	II	Lowest record	I	II	I	II	I	II	I	II	I	II
M	63 8	36 4	36 5	85 2	72 6	7 33	7 37	49 8	44 2	48 8	47 5	143 4	142 3
S D	17 7	27 0	12 3	28 6	13 4	0 04	0 04	6 0	4 1	5 3	6 6	5 9	4 5
S E M	6 3	6 6	3 7	11 7	4 3	0 01	0 01	2 1	1 2	1 9	2 0	[illegible] 1	1 3

analyzed The graphic resolution of the experimental curve was performed according to Sapirstein (21) The procedure of single injection clearance of inulin and its validity has been described in detail elsewhere (7)

The renal excretion of an oral sodium load was studied during standardized fluid expansion The healthy premature infants received breast milk diluted with 50% water by gastric tube During the first hour an amount *corresponding to 2% of the* body weight was given This was followed every 30 min by an amount of 0 5% of the body weight Infants with IRDS were given 5 5% *glucose* i v corresponding to 1% of the body weight during the first hour This was followed by 0 5% of the body weight per hour when feeding by mouth was impossible for any reason When a stable diuresis had been established 50–60 mmol Na /1 73 m² B S (2–3 mmol/kg/B W) was given orally in diluted breast milk Urine was collected by spontaneous voiding into urinary bags for a time period of 4–6 hrs from the time the sodium chloride was given The urinary excretion of sodium was *calculated as the average* hourly excretion per 1 73 m² B S

Inulin in blood was determined according to the Anthron method (15)

PAH in blood was determined according to Smith et al (24)

RESULTS

The clinical data of the 11 infants with IRDS are listed in Table 1 Apgar scores were 7 or more at 1 and 5 min of age except in case 2 who had scores of 3 and 5 respectively All chest roentgenograms showed a typical pattern of IRDS grade 1 according to the classification of *Prod hom et al* (19) Laboratory data of the diseased infants are summarized in Table 2 The mean of the lowest recorded P_{aO_2} is shown to give an impression of the degree of hypoxia the infants sustained Blood pressure recorded *in three cases* was within normal limits

Single injection clearance of inulin and PAH

Table 3 shows the summarized data of GFR C_{PAH} GFR/C_{PAH} and UNaV in IRDS infants and controls The table also includes gestational age post natal age at investigation and hematocrit values in the different groups

GFR was determined in 8 IRDS infants and 6 controls at a postnatal age of 37 and 33 hrs respectively There is no difference between the groups as regards postnatal age gestational age or hematocrit GFR is significantly higher in the IRDS group than in the controls 38 9 compared to 27 2 ml/1 73 *m²* B S */min* ($p<0\ 0005$)

A second determination of GFR was performed in 11 IRDS infants and 5 controls at a post natal age of 148 and 132 hrs respectively The two groups are comparable as regards gestational age postnatal age at investigation and hematocrit There is no significant difference in GFR between the groups with values of 30 9 ml/1 73 m² B S /min in the IRDS group and 38 3 in the control *group* When comparing the results within the IRDS group *the decrease* from 38 9 to 30 9 ml/1 73 m² B S /min is significant ($0\ 0125>p>0\ 01$) The increase in the control group from 27 2 to 38 3 ml/1 73 m² B S /min is also significant ($0\ 01>p>0\ 005$) These changes are graphically illustrated *in Fig 1*

C_{PAH} was determined in 8 IRDS infants and 5 controls at a postnatal age of 37 and 28 hrs respectively The two groups are comparable as regards gestational age and hematocrit but the control *group* is 9 *hrs younger* There is no

Table 1 *Clinical data and postnatal age at investigation in 11 infants with IRDS*

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Delivery	Treatment age in hrs	Postnatal age in hrs at invest	
1	f	36	2610	Vertex	CPAP 10 to 72 PEEP 72 to 192 Ampicillin from 36 Meticillin and streptomycin from 80 Pneumothorax at 29	48	144
2	m	32	1870	Vertex	PEEP 8 to 240 Lanoxin from 12 Ampicillin meticillin and streptomycin from 144	–	149
3	f	34	2500	Caesarean section	PEEP 4 to 96	35	114
4	m	36	2440	Vertex Preci pitated	PEEP 19 to 113 Lanoxin from 20 Ampicillin and meticillin from 80	–	157
5	f	34	2160	Caesarean section	CPAP 2 to 6 PEEP to 24 CPAP to 120+144 to 168 Pneumothorax at 142 Lanoxin from 27 to 190 Ampicillin and meticillin from 78	32	146
6	m	33	1750	Caesarean section	Oxygen by mask 2 to 48	36	176
7	m	34	2340	Caesarean section	CPAP 25 to 130	–	176
8	f	32	1730	Vertex	Oxygen by mask 3 to 100	29	145
9	m	33	1750	Caesarean section	CPAP 4 to 120	35	137
10	m	33	2070	Vertex	Oxygen by mask 2 to 170	50	147
11	m	36	2560	Caesarean section	CPAP 6 to 70	32	101

life IRDS was diagnosed clinically by signs of respira tory distress with retractions nasal flaring and expiratory grunting as well as by typical chest roentgenograms (20) All infants were placed in Isolette incubators with a rela tive humidity of 50 to 65% and ambient temperature between 31–34 C

The clinical data of infants with IRDS are shown in Table 1 Oxygen was administered to the infants with IRDS by face mask nasal continuous positive airway pressure (CPAP) nasotracheal CPAP or assisted ventila tion with positive end expiratory pressure (PEEP) in order to maintain an arterial P_{O_2} of 60 to 90 mmHg in the des cending aorta with the tip of the umbilical arterial catheter below the renal arteries 0 6 M $NaHCO_3$ was given to maintain the pH above 7 30 10% glucose supple mented with sodium and potassium was given i v in amounts of 60 to 120 ml/kg B W /24 hrs depending on the postnatal age The average hourly total sodium admini stration during the 24 hrs preceeding the first and second investigation was 3 and 1 8 mmol/1 73 m^2/h respectively The healthy pre term infants were fed breast milk in amounts of 60 to 120 ml/kg B W /24 hrs depending on the postnatal age Six of the sixteen infants received 10% glucose supplemented with sodium and potassium i v in the same amounts as for IRDS infants The average hour ly total sodium administration during the 24 hrs preceed ing the first and second investigation was 1 and 0 9 mmol/ 1 73 m^2/h respectively Five of the healthy pre term infants were delivered by caesarean section because of breech presentation Apgar scores were 8 or more at 5 min of age in all cases Oxygen was not administered to any of these infants The control groups of pre term infants were selected so that the gestational age and postnatal age would differ as little as possible from those of infants with IRDS

Informed parental consent was obtained in all cases studied The protocol was approved by the Committee of Ethics at Karolinska Institute

METHODS

Gestational age was determined using the method de scribed by Dubowitz et al (13)

Hematocrit was estimated in glass capillaries rinsed with heparin and centrifuged for 5 min at 12000×g

Sodium in serum and urine was analyzed by a flame photometer (Eppendorff)

Arterial blood pressure was measured with a Datascope Type P pressure module and read from a Datascope 850

Arterial blood samples for pH P_{CO_2} and P_{O_2} were col lected anerobically in disposable 1 ml syringes with heparin solution filling the dead space Analyses were performed on a Radiometer BMS 3 MK 2

Single injection inulin and PAH clearance Inulin and PAH was injected i v in a dose of 100 and 15 mg/kg B W respectively Calibrated glass syringes were used Samples of the test solutions were kept for determination of the concentration of inulin and PAH 0 4 ml blood was collected before the test solutions were given and then every third minute from 7 to 19 min and every fifth minute from 45 to 70 or 90 min The samples were collected in small test tubes rinsed with heparin and kept on ice until centrifuged The plasma was frozen and stored until

(U)NaV mmol/1 73 m B S /h			
(I)RDS	Control	IRDS	Control
5	7	7	5
9 5	1 4	7 0	1 9
[illegible]	1 1	2 9	0 4
1 0	0 4	1 1	[illegible]
3 6	34 6	33 9	34 0
1 5	1 3	1 7	0 7
0 7	0 5	0 6	0 3
5 8	32 0	149 9	134 0
7 3	7 3	13 7	20 9
[illegible]	[illegible]	5 2	9 3
4 4	53 0	41 6	49 4
3 2	5 2	8 3	5 8
1 4	2 0	3 2	[illegible]

0 0025) UNaV was determined a second time in 7 IRDS infants and 5 controls The groups are comparable as regards postnatal age at investigation gestational age and hematocrit The $UNaV_{IRDS}$ of 7 00 mmol/1 73 m² B S /h is significantly elevated as compared to 1 93 in the control group (0 0025>p> 0 0005) The decrease in the IRDS group and the increase in the control group is not significant In Fig 3 the values of UNaV in IRDS infants and controls are graphically illustrated Here the IRDS group is separated into two

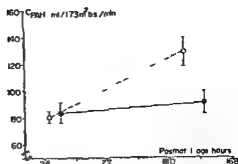


Fig 2 Development of C_{PAH} in controls (O) and IRDS infants (●) Range bars represent standard error of mean (S E M)

one group of infants treated with Lanoxin and/or Ampicillin (T) and one without such treatment (U) The difference between T and U is significant (0 05>p>0 025) There is also a significant difference in UNaV between untreated IRDS infants and controls (0 0025>p>0 0005)

No linear correlation was found between UNaV and $P_{a_{O}}$ in IRDS infants nor between UNaV and hematocrit In the control group there was a significant correlation between UNaV and hematocrit (r=0 61 0 025>p> 0 0125 regression line $y=-0\ 16x+10\ 24$)

DISCUSSION

The glomerular filtration rate in IRDS infants on the second day of life was found to be supernormal Later in the course of the disease GFR was slightly but not significantly lower than in the controls Previous studies of renal function in IRDS infants have given

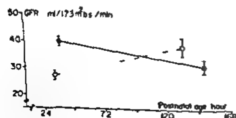


Fig 1 Development of GFR in controls (O) and IRDS infants (●) Range bars represent standard error of mean (S E M)

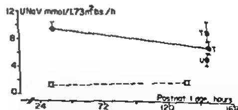


Fig 3 The average hourly sodium excretion following an oral sodium load in controls (O) and IRDS infants (●) *T* Values obtained in three infants treated with digitalis *U* Values obtained in four infants without digitalis Range bars represent standard error of mean (S E M)

Table 3 *Clearance data and average hourly sodium excretion UNaV in infants with IRDS and controls*

Gestational age and hematocrit in the different groups are also included

	GFR ml/1 73 m² B S /min				C_{PAH} ml/1 73 m² B S /min				GFR/C_{PAH} %			
	IRDS	Control	IRDS	Control	IRDS	Control	IRDS	Control	IRDS	Control	IRDS	Control
n	8	6	11	5	8	5	11	5	8	5	11	5
M	38 9	27 2	30 9	38 3	84 6	80 2	91 0	129 3	50	35	38	37
S D	4 7	4 4	8 0	8 1	22 2	10 7	29 7	25 4	17	8	16	13
S E M	1 7	1 8	2 4	3 6	7 8	4 8	9 0	11 4	6	4	5	6
Gestational age weeks												
M	33 8	34 2	33 8	34 4	33 8	34 4	33 8	34 4	33 8	34 4	33 8	34 4
S D	1 5	1 3	1 5	1 8	1 5	1 8	1 5	1 8	1 5	1 8	1 5	1 8
S E M	0 5	0 5	0 5	0 8	0 5	0 8	0 5	0 8	0 5	0 8	0 5	0 8
Post natal age hrs												
M	37 1	33 0	148 8	132 4	37 1	28 0	148 8	132 4	37 1	28 0	148 8	132 4
S D	7 7	3 7	27 6	24 7	7 7	2 1	27 6	24 7	7 7	2 1	27 6	24 7
S E M	2 7	1 5	8 3	11 0	2 7	0 9	8 3	11 0	2 7	0 9	8 3	11 0
Hematocrit %												
M	48 8	49 0	42 5	41 2	48 8	45 8	42 5	41 2	48 8	45 8	42 5	41 2
S D	5 3	4 4	6 6	6 7	5 3	8 6	6 6	6 7	5 3	8 6	6 6	6 7
S E M	1 9	1 8	2 0	3 0	1 9	3 8	2 0	3 0	1 9	3 8	2 0	3 0

difference in the C_{PAH} with values of 84 6 and 80 2 ml/1 73 m² B S /min respectively

A second determination of C_{PAH} was performed in 11 IRDS infants and 5 controls The groups are comparable as regards gestational age postnatal age at investigation and hematocrit The results for the IRDS group and the control group were 90 9 and 129 3 ml/1 73 m² B S /min respectively The difference is significant (0 025>p>0 0125) When comparing the results within the IRDS group there is no significant change in C_{PAH} whereas the increase in the control group from 80 2 to 129 3 ml/1 73 m² B S /min is significant (0 0025>p> 0 0005) The development of C_{PAH} in the two groups is graphically illustrated in Fig 2

GFR/C_{PAH} at the first investigation in IRDS infants is higher as a consequence of an elevated GFR and normal C_{PAH} in comparison to the controls i e 50% vs 35% (0 05>p> 0 025) At the time of the second investigation with values of GFR and C_{PAH} slightly below the controls there is no difference in GFR/C_{PAH} between the groups

No significant correlation was found between $P_{a_{O_2}}$ recorded at the time of the clearance study and GFR Higher values for r were obtained when relating the lowest recorded $P_{a_{O_2}}$ before the clearance study to GFR (r= 0 45 0 05>p>0 025) The same was found with the relation of $P_{a_{O_2}}$ to C_{PAH} but with higher significance (r=0 52 0 0125>p>0 01)

The average hourly excretion of an oral sodium load (UNaV)

UNaV was studied in 5 IRDS infants and 7 controls at a postnatal age of 36 and 32 hrs respectively UNaV in the control groups is of the same magnitude as has been reported earlier (3)

There was no increase of UNaV during the study This also accords with the study by Aperia et al (3) The $UNaV_{IRDS}$ amounts to 9 46 mmol/1 73 m² B S /h as compared to 1 37 in the controls The difference is significant (p<0 0005) There is a significant difference in hematocrit between the controls and IRDS patients i e 53% vs 44 4% (0 005>p>

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contradicting results Guignard et al (14) have shown that IRDS was accompanied by a marked decrease in GFR and C_{PAH} at a post natal age of 44 hrs (range 17–120 hrs) The de crease was most evident in the more severe cases On the other hand Siegel et al (22) reported that GFR was the same in IRDS as in healthy pre term infants In the present study a positive correlation was found be tween GFR and the lowest recorded arterial P_{O_2} before the clearance study It is there fore suggested that the effect of IRDS on renal function is most likely determined by the severity of the pulmonary disease which seems to be less pronounced in our study than that of Guignard et al (14) All of the patients survived and their roentgenograms showed grad 1 pattern according to the classification used by Prod hom et al (19) Higher gesta tional age and birthweight probably also indi cates a milder course of the disease

Different degrees of hydration may contri bute to the variations in GFR recorded in IRDS infants In our study the IRDS infants received sodium bicarbonate in rather high amounts during the 24 hrs preceeding the first study Fluid expansion of healthy pre term infants will result in a considerable increase in the GFR (17)

C_{PAH} in IRDS infants was similar in the first study but decreased in the second study in comparison to the controls In the newborn period, clearance of PAH cannot be taken directly as a measure of renal plasma flow as shown by Calcagno & Rubin (8) The low value of C_{PAH} may thus be either a sign of renal hypoperfusion or a delayed development of the tubular transport of PAH possibly in duced by hypoxia, as there is a significant positive correlation to the lowest recorded P_{aO_2} before the clearance study In IRDS infants the urinary sodium excretion was sig nificantly increased at both the first and second investigations in comparison to the controls Several factors may have contri buted to the relatively high sodium excretion In the present study the IRDS infants had a significantly higher sodium intake during the 24 hrs preceeding the first investigation and to a lesser degree also before the second inves tigation It is known that an increase of the daily sodium intake will increase the capacity to excrete an oral sodium load in infants (4) Glucose induced osmotic diuresis in the neo-nate has been shown to decrease proximal tubular sodium reabsorption (6) In our IRDS infants the daily fluid requirement was covered with a 10% glucose solution and this may therefore have contributed to the in creased sodium excretion

Ampicillin and lanoxin used in the treatment of IRDS infants are known to interfere with proximal tubular transport In the present study UNaV was significantly higher in infants treated with these drugs The effect is probably caused by lanoxin since it has been shown that digitalis can block the reabsorption of 35% of the filtered sodium (25)

Finally a direct effect of hypoxia on UNaV cannot be excluded since increased urinary sodium excretion has been demonstrated in hypoxic and asphyxiated animals (1 10 11 26) The amount of sodium that is reabsorbed determines renal oxygen consumption (12 26)

The danger of sodium administration has been widely discussed (2 23) In this study no infant died of cerebral hemorrhage and serum sodium concentrations were within normal limits It would appear that the amount of sodium used in these infants was not harmful The sodium was mainly given as bicarbonate and one study indicates that the natriuresis following a sodium load is higher when sodium is given as bicarbonate instead of as chloride (5) For reasons stated above it can thus be concluded that in premature infants with a moderate degree of IRDS that need correction of acidosis the daily sodium dose can be held relatively high

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CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTIONS

Virological and Clinical Studies on a Swedish Infant Population

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ABSTRACT Ahlfors K Ivarsson S A Johnsson T and Svensson I (Department of Clinical Virology and Department of Paediatrics University of Lund Malmo General Hospital Malmo Sweden) Congenital and acquired cytomegalovirus infections Virological and clinical studies on a Swedish infant population Acta Paediatr Scand 67 321 1978 —The study included two clinical materials First the frequency of cytomegalovirus and its clinical significance were studied among 661 Swedish children under one year of age admitted to a paediatric hospital Before the age of one week 4/326 (1%) children excreted virus At one month the frequency had risen to 6/52 (12%) and after this age the frequency was constant around 20–25% Sixty per cent of infants born to immigrants were infected after one month of age One of the four congenitally infected children had symptoms at birth followed by neurological sequelae The majority of the infections acquired at birth or in early infancy seemed to be subclinical and without sequelae Second a retrospective investigation of 18 695 children born during a six year period was performed Two cases of virologically confirmed congenital cytomegalic inclusion disease were found Regarding seven microcephalic patients in the retrospective study congenital CMV infection could be excluded in four cases In the remaining three cases the data did not permit any conclusions regarding the etiology

KEY WORDS CMV infections congenital neonatal postnatal microcephaly

Most children congenitally infected by cytomegalovirus (CMV) are asymptomatic at birth (8 15 19 20 22) Some of them however show clinical symptoms most frequently from the central nervous system (CNS) liver spleen and blood (7) that is the so-called congenital cytomegalic inclusion disease (CID) which sometimes is fatal According to Elek & Stern (3) the congenital CMV infection is the most common known viral cause of mental retardation which in some cases is combined with microcephaly obvious at birth or later (6 7 8 14 15 19 24) In contrast CMV infection acquired during the delivery or in early infancy is thought to be of little clinical significance (18) Thus for the prognosis it is essential to distinguish between congenital and neonatally acquired CMV infections The question whether the frequent finding of CMV among hospitalized infants had any causal relationship with the children s clinical symptoms and whether the virus infection was congenital or postnatally acquired provided the incentive for a more extensive investigation In addition a retrospective study of the occurrence of CID and microcephaly was made

MATERIALS AND METHODS

Study population The investigation was carried out in Malmö a city in the southern part of Sweden with one single hospital for somatic diseases serving 250 000 inhabitants Each year about 3 000 deliveries occur all of them in hospital There is one single institution for mentally retarded children in the city

Virus isolation Specimens from the patients were transported in Parker 199 with the addition of 1% albumin and antibiotics 0 2 ml of the samples was inoculated on



Table 3 *Clinical symptoms and occurrence of cytomegalovirus among 661 children studied*

Clinical symptoms	No patients in different age groups (months) <1	1–2	3–1?	Total
Infections				
Respiratory infections incl otitis	0/8	2/26	15/77	
Gastroenteritis	0/3	3/15	16/61	43/233
Urinary tract infections	0/11	3/14	3/8	
Meningitis septicemia	0/6	0/1	0/1	
Intrauterine infections	1/?	–	–	
CNS diseases				
Transient symptoms[b]	0/1	0/?	3/8	
Lasting symptoms	–	–	0/2	4/16
Malformations[d]	0/1	1/1	0/1	
Transient neonatal problems				
Aberrant birth weight incl preterm	1/83	–	–	
Minor complications	1/58	–	–	3/313
Major complications[f]	1/17?	–	–	
Failure to thrive	1/ 0	1/8	–	2/ 8
Malformations CNS excluded	0/18	0/6	–	0/24
Diverse diagnoses	0/11	4/19	5/17	9/47
Total	5/394	14/92	42/175	61/661

No of virus positive patients/total no of patients investigated

Convulsions due to fever hypoglycemia cerebral hemorrhage hygroma

Cerebral palsy mental retardation

Microcephalus myelomeningocele

Stridor hypothermia cyanosis thirst fever myoclonic tremor cephalhematoma maternal diabetes mellitus

Hyperbilirubinemia asphyxia idiopathic respiratory distress syndrome Mb haemolyticus neonatorum pulmonary atelectasis hyperviscosity syndrome hypocalcemia transfusion foetomaternalis cum anaemia gravis

Teratoma lymphangioma cavernosum hemihypertrophy trisomia pylorostenosis cheilognathopalatoschisis

Obstipation invagination aspiration eczema breath holding spells morbilli phlegmona causa socialis

The corresponding figures for the remaining children mainly with native born mothers were 5/373 (1%) at ≤4 weeks and 41/241 (17%) at 1–12 months

The four patients with virus demonstrated within the first week of life regularly excreted virus in the urine until 2½ years of age At 3–4 years three of them still were virus positive Among 23 followed up patients with viruria detected after one month of age the following numbers were positive at their last control at one two and three years 7/7 6/6 and 9/10 respectively (Table 2) Up to two years of age 92/94 (98%) urine samples and 49/58 (84%) throat samples from the above mentioned 27 patients were virus positive

Patient material I clinical findings

Clinical symptoms of the 661 children at the detection of CMV excretion The patients have been divided with regard to their symptoms into six main groups According to Table 3 the number of CMV excreters out of the total number of patients in the different symptom groups was 43/233 with different kinds of infection 4/16 with CNS diseases 3/313 with transient neonatal problems 2/28 with failure to thrive 0/24 with malformations and 9/47 with diverse diagnoses Generally the proportion of excreters increased with age independently of the symptoms Four patients had obvious splenomegaly none had significant enlargement of the hepar

Case reports of the four children with CMV excretion detected within one week after birth One of the four patients had CID with a low birth weight (≤2500 g) and two others had low birth weight one of whom also had hyperbilirubinemia The fourth infant was born to a mother who had CMV-disease in mens *VII* (Table 4) At ≥3 years of age only the patient with CID (Case 1) showed neurological sequelae Coexistent congenital infection caused by listeria toxoplasma or rubella virus was excluded by laboratory tests in this case This patient and another one (Case 3) had positive CMV IgM reaction in cord serum Only Case 3 was controlled with the Latex RF Reagenz

Follow up investigation of the children with CMV excretion detected at 1–12 months of age Forty nine out of 56 virus excreters could be followed until 1–4 years of age Forty four of them were in good health at the final examination Five patients all children of native born mothers had some permanent disorder

Table 1 *The frequency of cytomegalovirus in urine samples obtained from children on admission to a paediatric hospital*

Age of the patient		Total no of patients investigated		No of patients with viruria	
≤1 week		326	394	4 (1%)	5 (1%)
≤2 weeks		27		0	
≤3 weeks		24		0	
≤4 weeks		17		1	
≤5 weeks	1 month	14	52	1	6 (12%)
≤6 weeks		14		2	
≤7 weeks		11		0	
≤8 weeks		9		1	
≤9 weeks		4		2	
2 months		40	215	8 (20%)	50 (23%)
3 months		27		8 (29%)	
4 months		23		5 (22%)	
5 months		14		3 (21%)	
6 months		33		8 (24%)	
7 months		12		3 (25%)	
8 months		16		3 (19%)	
9 months		20		4 (20%)	
10 months		19		5 (26%)	
11 months		11		3 (27%)	
Total		661		61	

the day of sampling on two tissue culture tubes containing human foetal lung fibroblasts grown in Eagle s MEM with 10% foetal calf serum and maintained in Eagle s MEM with 3% foetal calf serum and antibiotics The cultures were controlled once a week during a four week period and then discarded Growth of CMV was identified in two ways by indirect immunofluorescent test performed with paired acute and convalescent sera from a patient with a clinically and laboratory confirmed CMV infection and by its failure to replicate in simian epithelial cells

Serological tests Cord sera and maternal sera drawn during the pregnancy and at the delivery were collected as a rule However in the present paper serological results will be discussed only if they are of special interest Complement fixation (CF) test was performed by a microtiter technique using 4–6 units of ultrasonicated and heated (56 60 min) CMV AD 169 cell antigen CMV IgM antibodies were estimated by means of their ability to react with intranuclear CMV inclusions in an indirect fluorescent antibody test which will be described in detail later on IgM positive sera were tested for the occurrence of IgM anti IgG with the Latex RF Reagenz Behringwerke Germany (17)

Patient material I virus isolation During a period of 14 months November 1971–January 1973 patients admitted to the paediatric hospital on week days were investigated on admission with regard to the excretion of CMV in the urine In the beginning all patients under one year of age were studied During the last seven months of the period the investigation was limited to infants ≤3 months old Altogether 754 patients were studied but 93 of them the majority <1 month old were excluded because of degeneration of the cell cultures The age distribution of the 661 patients included in the material is shown in Table 1 Of the patients who showed CMV-excretion 27/61 (44%) could be followed up at varying intervals as regards urine and/or throat samples from one to four years of age (Table 2)

Patient material I clinical investigation At the clinic the children were primarily examined without any special regard to CMV infection The clinical symptoms of the patients while in hospital are shown in Table 3 Patients with CMV-excretion were called for further controls Out of 61 excreters 53 (86%) were followed until ≥1 year of age 44 of them until 2–4 years Special attention was paid to malformations and to the psychomotor development Children with CMV-excretion at birth were examined by an audiologist at 3–4 years

Patient material II In the retrospective investigation 18 695 children born in Malmo during a six year period 1970–1975 were studied with regard to the occurrence of congenital CID and CMV-caused microcephaly Microcephaly has been defined as a head circumference less than the mean value of normal children minus 2 S D (16)

RESULTS

Patient material I laboratory studies

Totally 61/661 patients excreted CMV on admission to the hospital Five out of 394 (1%) children ≤4 weeks old were virus positive four of them within one week after birth and one at the end of the fourth week while 6/52 (12%) were excreters at one month and 50/215 (23%) at 2–12 months of age (Table 1) The difference before and at one month of age is statistically significant ($p<0.01$) Among a total of 47 children born to mothers who had immigrated from the Central or Southern Europe 0/21 showed viruria at ≤4 weeks of age compared to 15/26 (57%) after this age

Table 2 *The result of virus isolation studies at the latest control*

Age at the detection of CMV excretion	No patients virus positive/ no tested at different years of age			
	1	2	3	4
<1 week			2/2	1/2
1–12 months	7/7	6/6	9/10	0/0

able 6 *Case reports of two patients with congenital cytomegalic inclusion disease*

atient	Sex	Gestational age birth weight	Symptoms at birth	Viruria at <week of age	CMV IgM in cord serum	Rheuma factor test on cord serum	Development at ? years of age
:ase 1 Identical to Case 1 in *Table 4*	M	41 weeks 2 500 g	CID	+	1/32	ND	Sequelae
:ase 17 3 10 30 ML	M	37 weeks 2 690 g	Thrombocytopenia *hepatosplenomegaly*	+	1/64	<1/?	Normal *development*

:hildren and the institute of pathology (Table ; and 7) Patients with slight manifestations which also may be compatible with other dis eases than CID were not considered All mic rocephalics in the *retrospective study* ex cept Case 11 were born without signs of in fection Three of them were diagnosed as microcephalic at birth four of them acquired their microcephaly later on CMV could not be isolated in 3/7 cases tested before six months of age and in 5/7 cases tested CMV IgM anti bodies could not be demonstrated in cord se rum In Case 10 a persisting high rubella hemagglutination inhibition titer was demon strated The CID patients and the micro- cephalics *except* Case *15* were all born to na tive mothers

DISCUSSION

The present investigation shows a marked dif ference in the virus excretion before and after one month of age One per cent of the chil dren under one week of age excreted CMV compared to 12% at the age of one *month* which is a statistically significant difference ($p<0.01$) The present *material* does not per mit any analysis of the frequency of CMV infections *during the* 2nd–4th week of age since the number of patients at this age was relatively *small with* only one excreter in the fourth week (Table 1) With regard to statisti cal reasons it may be assumed that the majori ty of the patients with CMV uria demonstrated after one month of age had a postnatally ac quired infection The study confirms the con clusion drawn by Reynolds et al (18) that only isolations performed before 2–3 weeks of age are diagnostic for congenital infection The *figure of one per cent of congenital infections* in the present selected material is of the same size as the 0 5–1 5% in unselected materials given by Reynolds et al (19)

In the present study the number of excreters stabilized around 23% in the third month of age which is an average figure compared to those previously reported *from* Germany (20–30%) and Finland (23%) while in Denmark (5–8%) England (9–10%) and USA (10–13%) the figures are lower (1 9 10 13 21) Based on CF tests performed on children 6–12 months old the frequency of CMV infections in different geographical areas including Den mark England and USA varies between 20–35% (1 2 4) The present frequency was in fluenced by the high *number of positives* among children of immigrants Thus 15/26 (57%) *were positive* among these children >4 weeks old but only 41/241 (17%) among the re maining infants of the same age This finding is in agreement with those of Li et al (11) and Stoll (23) who ascribed their observations to socio-economic conditions

The investigation confirms earlier reports that prolonged virus *excretion may follow* both congenital and postnatally acquired CMV infections (24) *Considering* the high frequency (98%) of positive isolations from urine samples *among* patients followed up to two years of age failure to isolate CMV before this

Table 4 *Case reports of four patients with congenital cytomegalovirus infection*

Patient	Sex	Gestational age birth weight	Symptoms at birth	Viruria at <1 week of age	CMV IgM titer in cord serum	Rheuma factor test on cord serum	Development at 3–4 years of age
Case 1 72 01 15 MR	M	41 weeks 2 500 g	Dysmature petechiae thrombocytopenia hepatosplenomegaly paraventricular calcifications	+	1/32	ND	Strabismus bilateral surditas infantil autism
Case 2 71 11 05 MS	F	35 weeks 2 400 g	Hyperbilirubinemia	+	<1/4		Normal development and hearing
Case 3 72 09 08 CB	F	40 weeks 2 200g	No symptoms	+	1/32	<1/2	Normal development and hearing
Case 4 72 12 05 BK	M	40 weeks 3 800 g	No symptoms Maternal CMV	+	<1/4		Normal development and hearing

* Not done

(Table 5) In three cases the disease was hereditary In a fourth case with the disorder persistent since birth a virus isolation attempt performed neonatally was negative In the fifth child who had a CF negative mother persisting EEG changes started after hypoglycemic convulsions CMV IgM antibodies could not be demonstrated at birth in any case

Patient material II clinical and laboratory findings

In the retrospective investigation two patients with obvious symptoms of CID one of them identical to Case 1 in patient material I and *seven patients with microcephaly were traced* in the diagnosis registers of the pediatric clinic the institution for mentally retarded

Table 5 *Follow up investigation of 49 children with CMV excretion detected at 1–12 months of age Case reports of five patients with permanent disorders*

Patient	Sex	Mother	Condition of patient	Age	Viruria	CMV CF titer	CMV IgM titer	Comments
Case 5 72 01 04 JL			Werdnig Hoffmann s disease diagnosed at 1 year of age	Birth 2 months	ND +	ND ND	<1/4	Hereditary disease
Case 6 71 10 15 PB	M		MB Down	Birth 7 months	ND +	ND ND	<1/4	Chromosomal aberratio
Case 7 71 07 15 HP	F		Hereditary malformations incl the ears Impaired hearing	Birth 8 months	ND +	ND ND	<1/4	Hereditary disease
Case 8 72 05 12 TE	M		Shunt operated for hydrocephalus at 2 months of age	Birth 1 week 1 month	ND – +	ND ND ND	<1/4	Cong CMV inf not probable because of neg virus isolation at 1 week
Case 9 71 06 15 CB	F	CMV CF titer <1/5 at the delivery	At 9 months hypoglycemic convulsions resulting in persistent EEG changes	Birth 10 months	ND +	<1/5 ND	<1/4	Cong CMV inf not lack of maternal CF antibodies at the delivery

omments
ersisting high rubella hemaggl antibody titer Probably cong rubella inf
n elder brother also microcephalic Neonatally hrombocytopenia and myoclonic tremor Cong CMV inf not probable because of lack of maternal CF antibodies at the delivery and negative virus isolation at 2 weeks
ervus opticus coloboma Cong CMV inf not probable because of negative virus isolation at 5 months
lulticystic kidney Cong CMV inf not probable because of negative virus isolation at 1 month
lead 1 day old Autopsy Myelomeningocele microgyria Cong CMV inf can not be excluded
tatus epilepticus at ? years Cong CMV inf can not be excluded
At 3 weeks resuscitation after unconsciousness Op bilateral hygroms Cong CMV inf can not be excluded

port from Finland (5) also confirms this finding

Concerning the five congenitally infected children (Cases 1 2 3 4 and 17) only two (Cases 1 and 17) were born with obvious clinical symptoms one of whom (Case 1) had severe neurological sequelae at the follow up The combined data of three previous prospective studies show that two of 51 congenitally infected infants among a total of 4830 tested by virus isolation neonatally had hepatomegaly alone and another had intracranial calcifications (15 20 22) Before the isolation of virus there was no clinical suspicion of congenital CMV infection in any of the cases Serious neurological disturbances developed in one of the two children having hepatomegaly as well as in the infant with intracranial calcifications and in one free of symptoms at birth In summary most congenitally infected infants are free of symptoms at birth late sequelae may follow in either symptomatic or asymptomatic excreters and symptoms at birth are not necessarily followed by sequelae Although no statistically significant conclusions can be drawn from the present study the findings are in agreement with the investigations quoted above

In the retrospective study where patients with microcephaly or congenital CID were chosen as index cases of congenital CMV infection seven microcephalics (Cases 10–16) and two cases of congenital CID (Cases 1 and 17) were found among 18 695 patients studied Congenital rubella infection was on serological grounds a probable etiology of the microcephaly in one case no 10 Congenital CMV infection was not probable in Cases 11–13 because of negative virus isolation attempts during the first months of life In cases 14–16 the data did not permit any conclusions The frequency of CMV caused microcephaly should accordingly be ≤3/18 000 (≤0 2‰) which is comparable to the combined data 3/15 775 (0 2‰) found among prospectively studied patients evaluated by virus isolation alone or by a combination of IgM antibody determination and virus isolation neonatally (8 15 19 20 22) It might be mentioned that the overall risk for neurological disturbances according to the same data is 14/15 775 (0 9‰) with hearing impairment as dominating symptom The two patients with congenital CID were diagnosed both by virus isolation and by demonstration of CMV IgM antibodies in cord serum CMV IgM antibodies could not be shown in cord serum in any of the five cases of microcephaly tested The role of CMV IgM antibodies for the diagnosis of congenital CMV infection will be discussed in a paper under preparation

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Table 7. *Retrospective investigation. Case reports of seven patients with microcephaly*

Patient	Sex	Mother	Gestational age, birth weight, head circumference	Age, head circumference	Age	Virus urina	CMV CF titer	CMV IgM titer
Case 10 73 11 11 FRA	M	Normal pregnancy	38 week 1800 g 30 (32)[a] cm	2 years 43 (46) cm	Birth 10 months	ND[b] ND	1/40 ≤1/4	<1/4
Case 11 75 07 27 AÅ	M	Normal pregnancy CMV CF titer <1/5 in the delivery	40 week 2960 g 32.5 (32) cm	8 months 42 (43) cm	Birth 2 weeks	ND –	<1/5	<1/4
Case 12 70 05 06 FÖ	M	Normal pregnancy CMV CF titer 1/80 at the delivery	43 week 2210 g 31 (32) cm	14 months 41 (44) cm	5 months	–	<1/5	
Case 13 70 12 18 AJ	F	Normal pregnancy	35 week 2360 g 30 cm	6 years 47.5 (48) cm	1 month 6 months	– ND	1/16 1/4	
Case 14 72 08 21 AP	F	Normal pregnancy	38 week 2200 g 31 (32) cm		Birth	ND	1/40	<1/4
Case 15 72 12 30 AA	M	Abortus imminens in early pregn	40 week 2830 g 33 (32) cm	4 years 47 (48) cm	Birth	ND	1/80	<1/4
Case 16 74 12 19 POÅ	M	Normal pregnancy	39 week 3500 g 37 (32) cm	2 years 43 (47) cm	Birth	ND	1/40	<1/4

[a] The mean value of the head circumference of normal children minus 2 S.D.
[b] Not done.

age may be regarded as a strong evidence against previous infections, congenital or postnatally acquired.

With regard to the finding of a protracted, easily demonstrable virus excretion among the infected children and a constant virus frequency after two months of age it seems reasonable to assume that few new CMV infections occurred after that age. Otherwise an accumulation of virus positive patients with increasing age could have been expected. This assumption is supported by a recent report by Granström et al. (5). The appearance of infections 1–2 months after birth makes a transmission from the mother probable.

If the above mentioned assumption is correct clinical symptoms of acquired CMV infections should be expected at 1–2 months of age. There was, however, no statistical difference in the frequency of CMV excreters among patients 1–2 months old with infections (8/56) compared to patients with the same symptoms but 3–12 months old (34/147) ($p>0.05$). Nor was there any statistical difference in the frequency of CMV excreter among patients with infections 1–12 month old (42/203) and patients of the same age but with other diseases (14/64) ($p>0.05$) (Table 3). Regarding the prognosis after neonatally acquired infection five patients with CMV detected after one month of age were found to have persistent disorders (Table 5). Only in one case, no. 9, a postnatally acquired infection theoretically may have caused the permanent disorder. There was no support for congenital infection in any case. Altogether the data presented indicate that the majority of the acquired CMV infections were without obvious acute clinical symptoms and without sequelae. The subclinical character is supported by previous observations that children neonatally infected by blood transfusion (12) or by CMV excreting mothers (18) do not show any clinical symptoms. The recent re

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CONGENITAL DISLOCATION OF THE HIP IN NORWAY

Late Diagnosis CDH in the Years 1970 to 1974

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ABSTRACT Bjerkreim I and Årseth P H (Sophies Minde Orthopaedic Hospital University of Oslo Oslo Norway) Congenital dislocation of the hip in Norway Acta Paediatr Scand 67 329 1978 —A survey of 274 late detected cases of CDH born in the years 1970–74 is presented The incidence of late cases in southeast Norway was calculated to 2 2 per 1 000 live births The hips of all patients were examined at birth the majority by trained pediatricians without disclosing any hip affections 86% were females Only 6 9% were delivered in the breech position One-half of the patients had luxation (20%) or subluxation (30%) the rest had dysplasia without dislocation In 19% both hips were involved The low incidence of breech presentations in late CDH-cases compared with the incidence among neonatal cases (15 7%) point to some etiological differences It seems that we in Norway have two types of CDH one caused by joint laxity and detectable at birth and another not present at birth with progressive dysplasia of the hip and dislocation developing during the first year of life

KEY WORDS Congenital dislocation of hip late diagnosis

In a previous study of 799 late-detected cases of congenital dislocation of the hip (CDH) we found the incidence to be 2 per 1 000 live births in Norway in the years 1960 to 1969 (4) During the same period 1 183 newborns with a positive dislocation test (Ortolani test) were treated and the incidence of neonatal CDH was calculated to be 8 per 1 000 Thus a total of 1% of all live births were treated and this is about 10 times the incidence estimated by Getz (13) when the diagnosis was made in the walking age group The Malmo group (10 17) have suggested that the relatively high incidence of late congenital dislocation of the hip could be due to inadequate examination techniques at birth Our earlier data do not confirm this interpretation

The present paper is a continuation of our investigation of late-detected CDH to see if increased alertness and further experience in diagnosing the disorder at birth have reduced the frequency of late cases

MATERIAL AND METHODS

The total material consists of 274 late-detected cases of CDH born in the years 1970 to 1974 inclusive All cases have been treated at Sophies Minde Orthopaedic Hospital during the years 1970 to 1976 For calculation of the incidence of late cases only those born in five counties in southeast Norway (Akershus Oslo Hedmark Oppland and Buskerud) a total of 222 were included

The investigation is based on hospital records and the X rays of all patients Cases without obvious pathological findings on X rays were discounted

RESULTS

Of the total number of patients 86 1% were females and 13 9% were males Eighteen (6 9%) were born in the breech position (Table 1) Half of the patients presented with luxation or subluxation and the other half with

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The investigation is based on hospital records and the X rays of all patients Cases without obvious pathological findings on X rays were discounted

RESULTS

Of the total number of patients 86 1% were females and 13 9% were males Eighteen (6 9%) were born in the breech position (Table 1) Half of the patients presented with luxation or subluxation and the other half with

Table 1 *Late diagnosis CDH patients born in the years 1970 to 1974 and treated at Sophies Minde Orthopaedic Hospital Oslo*

	Sex		Total	Breech presentation
	Female	Male		
No	236	38	274	18/244
Per cent	86 1	13 9	100	6 9

dysplasia without dislocation Nearly 50% had a left sided affection and in 19% both hips were involved (Table 2) Mean age at start of treatment decreased from 9 6 months for patients born in 1970 to 6 0 months for those born in 1974 (Table 3)

The incidence of late diagnosis CDH is given in Table 4 222 children (2 2‰) out of all live births in the Oslo region were treated for CDH diagnosed after the newborn period Thirty eight (0 4‰) had luxation 64 (0 6‰) subluxation and 120 (1 2‰) had dysplasia without dislocation

Most of the cases (65%) were detected at the child welfare clinics Twenty four (8 8%) of all late cases had concomitant foot anomalies and 4 had other congenital malformations

DISCUSSION

The investigation shows that we yearly still treat about 55 new cases of late detected CDH this in spite of the fact that the dislocation test of Le Damany & Saiget (16) Ortolani test was introduced in Norway as early as 1954 (19) and despite the fact that all newborns since 1967 have had their hips examined at birth

The distribution by sexes 86% females in the present investigation is in accordance with our previous series of late detected case most other series The proportion of bre presentations is as we have found befor lower than in most investigations, 6 9% (the neonatal cases however 15 7% were bo by the breech (4) This difference may po to some etiological differences among earl and late diagnosis CDH as discussed previou ly (5)

About one half of the patients had luxati (20%) or subluxation (30%) the rest had t mildest degree of hip malformation dyspla without dislocation The left side was mo often affected than the right as found in m series of CDH but the percentage of bilate affections was low The high proportion dysplasia and the low mean age at diagno may be the reasons for this Of those hav luxation or subluxation the percentage of lateral cases was 27

The incidence of late diagnosis CDH v found to be 2 2‰ with a variation fr 1 5 to 2 9‰ through the five years inve gated There was no decreasing tenden Late cases thus are presently just as frequ as during the years 1960–69

The hips of all our late cases were exami at birth many by trained pediatricians 15 years of experience Twentyfour pati had concomitant foot deformities and the gr majority of these had also had their h examined by an orthopaedic surgeon dur the first 3 days of life without disclosing hip affections

The frequency of *neonatal* CDH has be fairly constant during the last 5 years thus total incidence of CDH in Norway is about same as in the 1960 s and we treat about times the previously accepted incidence

Table 2 *Degree of hip deformity and affected side in late diagnosis CDH patients*

	Degree of hip deformity			Affected side		
	Luxation	Subluxation	Dysplasia	Right	Left	Both
No	55	78	141	87	134	53
Per cent	20 1	28 5	51 4	31 8	48 9	19 3

Table 3 *Mean age at diagnosis in CDH patients*

	1970	1971	1972	1973	1974
Mean age (months)	9 6	8 5	8 8	7 0	6 0

1 2‰ found by Getz (13) His calculations were however based on questionnaires to public health officers and the figures must be interpreted as minimum values Some regional but more reliable investigations have shown an incidence of CDH of 3 to 5‰ in Norway at a time when no treatment was given to newborns (3 18) and before examination of infants hips became a routine We also know that a great number of children with lesser degrees of CDH was not detected in former days The dysplasia gave rise to no symptoms until complicating osteoarthrosis developed in adulthood

We have good reason to believe that nearly all cases of CDH are detected during the first year of life Cases not detectable or missed at birth also those having minor hip deformity subequently become detected during infancy in the child welfare clinics In Norway hip examination is part of a systematic routine examination of all infants The scheme was introduced 15 years ago and as nearly all late cases of CDH whether dislocated or not have a positive abduction test detection is easy

Investigations from Norway have shown that congenital dysplasia of the hip joint is the main cause of osteoarthrosis of the hip counting for about 50% of all cases (6 12 14) The incidence of coxarthrosis was calculated in Sweden to be 3 4% in the age group above 55 years (8) The fact that we now treat 1% of newborns and infants for CDH is therefore really not surprising

Our series of 1 183 neonatal cases have had a careful follow up with clinical and radiographical examination at regular intervals (4) The study of all X rays revealed that about 50% of the cases showed varying degree of residual hip joint dysplasia in the early course after the pillow treatment and 25% were given additional treatment In the most reliable diagnostic group those with a distinctly positive dislocation test in the obstetric units and when examined by an orthopaedic surgeon as well as in the other diagnostic groups the percentage of patients showing radiographic signs of dysplasia was high The registered pathological X ray findings were remnants of the disease for which the children had been treated and not complications of the treatment We seldom if ever see complications of the pillow treatment though the pillow as other forms of splintage does fail in some cases We have not seen ossification defects as found by Fellander et al (9) in a great number of cases treated with the von Rosen type of splint neither have we seen facial paralysis as described by Beddow (2) using the same type of splint We also treated 163 newborns who only had a click but no instability of the hip joint (4) These hips were believed to be normal but the children were followed up as well None had residual dysplasia or other signs of damage to the hip joint Investigation of long term results of our treatment of neonatal CDH are not yet concluded but judged from the preliminary results the

Table 4 *The incidence of late diagnosis CDH in five counties in southeast Norway*

Years	Live births No	Late-diagnosis CDH							
		Luxation		Subluxation		Dysplasia		Total	
		No	Per 1000	No	Per 1000	No	Per 1000	No	Per 1000
1970 to 1974	9968	38	0 4	64	0 6	120	1 2	222	2 2

hips will certainly turn out to have become radiographically normal in more than 95%

Geographical variation in the incidence of CDH is well known However the difference between Norway and Sweden seems surprising In Malmo 12 to 20 per thousand of newborns were treated for CDH during the past 10 years (10) but few late cases at least with dislocated hips In Uppsala Sweden on the other hand they treated 1 9 per thousand live births for late diagnosis CDH in spite of a high incidence of neonatal cases (15) There may be several explanations for the difference in incidence of late cases in different regions It seems that in Norway we have two types of CDH corresponding to the classification of Wynne Davies (20) one caused by joint laxity and detectable at birth and the other probably not present at birth with a progressive dysplasia of the acetabulum and dislocation developing during the first months of life In Malmo only the first type may exist Another possibility is differences in screening for CDH in infancy A third reason seems to be differing opinion as to the normal variation of the radio graphic picture of hips of infants and children Futhermore if radiographs are taken with the hips in full inward rotation a subluxation may be reduced and the result wrongly interpreted as normal

There can hardly be any discussion that diagnosis and treatment of CDH in newborns is of great importance However we have to face the fact that many cases are not detect able at birth and that hip joint dysplasia and dislocation may develop in the first few months of life Therefore the hips of all infants should be examined at regular intervals i e at 2 4 and 6 months of age Thus the great majority of missed cases will be detected early and have the opportunity of normal development if properly treated

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The Editor has asked Assist Professor Kurt Palmen to comment on this article

n their article Congenital dislocation of the hip in Norway Bjerkreim & Årseth (1) give an account of the late diagnosis cases of CDH during 1970–76 treated at the Orthopaedic Clinic in Oslo Compared with the earlier figures from the sixties there is still the same high frequency of 2 2‰ of the liveborns in spite of the fact that all newborns are examined as to the hips

In Sweden we have a much lower frequency of such cases In 1973–76 at all our orthopaedic clinics about 200 cases were treated that is 0 5‰ of the liveborns (5) In some centres we had very low figures e g in Malmo only 0 07‰ (3)

The authors discuss the possible reasons for this difference and they have an especially controversial opinion in relation to the Malmo group who have in articles in this journal and in Lakartidningen been very critical against the CDH diagnosis and treatment in Norway

There are several reasons for the different views of the CDH in Norway and Sweden

Both the very low frequency of late diagnosis dislocations and the very good treatment results in Malmo are however not representative for the whole of Sweden We still have problems with CDH as in Norway but we believe this mainly depends on the organization of the examination and the treatment programs In Gothenburg for example we have found better results with the von Rosen splint than with the Frejka pillow according to the preliminary results of a follow up (4)

The primary results of the treatment are everywhere very good and this is not surprising as most of the hip joint laxity cases will become normal without any treatment In the treated cases however it is not unusual in the X ray films to find a delay of the ossification centre of the femoral head and even small differences in the acetabular margin usually called dysplasia a diagnosis with inexactly defined criteria In such cases it is at 4 to 6 months up to a subjective judgement to evaluate the need for additional treatment Will the hip development be normal or is it necessary to add a plaster bandage for a few months? This affects the figures of the treatment results

As to the frequency of hip joint laxity in newborns the figures in Norway and Sweden are about the same but there is a great variation between different obstetric clinics Bjerkreim (1) found in Oslo 8‰ and Cyvin (2) in Trondheim 19‰ Fredensborg (3) reported 9 3‰ in Malmo and in 1974 Palmen (5) has found at two thirds of the Swedish obstetric clinics with 60000 newborns a frequency of 11 7‰ with variations up to more than 20‰ To some extent the high figures are attributable to overdiagnosis due to less experience of the examiners

Differences in the CDH have been found in several countries not only as to the frequency and the birth characteristics but even as to the severity of the cases probably according to genetic factors Until we have got further reliable knowledge of the CDH in our countries we have to accept that there may be some differences in Norway and Sweden However we have a unique opportunity to study these problems all newborns can be examined and we have an excellent opportunity to organize the treatment and make follow ups

Friendly cooperation between the orthopaedic surgeons and the paediatricians working with the CDH in our countries would be very useful in solving the problems and better than futile criticism

Kurt Palmen

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PLASMA FREE AMINO ACID LEVELS DURING THE INITIAL REHABILITATION OF PROTEIN ENERGY MALNUTRITION WITH PROTRACTED DIARRHOEA USING A FREE AMINO ACID–GLUCOSE DIET

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ABSTRACT Lindblad B S Rahimtoola Razia J Hafiz ur Rehman Shamim Ahmad S Fancy Khurshid Singha Lily and Sajjad Hussain S (Department of Paediatrics of Karolinska Institutet at St Goran s Children s Hospital Stockholm Sweden and Department of Paediatrics of Jinnah Postgraduate Medical Centre Karachi Pakistan) Plasma free amino acid levels during the initial rehabibtation of protein-energy malnutrition with protracted diarrhoea using a free amino acid–glucose diet Acta Paediatr Scand 67 335 1978.—This study intends to assess by ion-exchange chromatography of free amino acid levels of peripheral blood plasma the amino acid absorption of severely growth retarded infants with protracted diarrhoea during the initial period of rehabilitation Eleven infants from a very low socio-economic group of a developing country with nutritional marasmic growth retardation and prolonged diarrhoea were treated for a period of 10 days with a commercially available free amino acid–glucose diet (Vi onex® Pfrimmer Co Erlangen Germany) Excessi e hyperprolinaemia characterized the plasma aminogram before treatment After initial rehabilitation with this diet the plasma analyses showed very low branch-chained and cystine levels and marginally high alanine glycine and proline levels It seemed that the free amino acids could not be absorbed quickly enough to meet with the high supply of glucose Furthermore this investigation supports the assumption that cystine is an essential amino acid in malnourished infants In spite of normal or high human growth hormone levels somatomedin was not detectable in pooled samples from these severely growth retarded infants

KEY WORDS Elemental diet protein-energy malnutrition diarrhoea malabsorption amino acids plasma human growth hormone somatomedin

Low levels of branch-chained amino acids in blood plasma are a constant feature of protein energy malnutrition It has been suggested (17) that leucine itself which induces insulin release by a different mechanism from that of glucose and arginine (15) acts as a physiological insulin feed back regulator But the albumin synthesis of the liver is seriously affected by low branch-chained amino acid levels of plasma (32) and rehabilitation should therefore aim to normalize as quickly as possible the distortion of the plasma homeostasis of free amino acids

In protein energy malnutrition there is reduced pancreatic enzyme secretion (8) and atrophy of surface jejunal and colonic epithelium (13 45 56) with reduced dipeptide hydrolase (12 33) and disaccharidase activity (10 30) There are also indications of an increased risk of transfer of intact protein molecules across the atrophic mucosa (61) speaking in favour of a hypoallergenic diet during

Table 1 *Clinical data*

MAC = midarm circumference TB = pulmonary tuberculosis Weights are before i v fluid therapy of dehydration and electrolytical imbalance and after 10 days dietary treatment (an additional week in case 11)

Case	Age (months)	Height (cm)	MAC (cm)	Weight in g Before	Weight in g After	Diagnosis
1	1 5	48	6	2 720	3 320	Diarrhoea
2	2	53	8	3 060	3 720	Diarrhoea
3	5	56	7	3 240	3 670	Diarrhoea + TB
4	6	56	7	3 350	4 020	Diarrhoea + TB
5	6	61	8	3 240	3 320	Diarrhoea + TB
6	7	58	8	2 750	3 780	Diarrhoea + TB
7	7	61	7	4 380	5 300	E coli OB/126
8	12	69	7	4 820	5 900	E coli OB/086 + TB
9	18	74	9	6 350	6 580	Diarrhoea + rickets
10	18	74	13	6 380	6 800	Diarrhoea + oedema
11	24	74	8	5 000	7 700	TB

the rehabilitation of severe malnutrition Studies in semi starved rats and guinea pigs suggest an increased absorption of amino acids and glucose in malnutrition (23 27 53) and it seems therefore that the possibility of peroral amino acid and monosaccharide feeding should be more extensively investigated

Free amino acid diets as developed and investigated by Greenstein et al (25) were chosen in the present study rather than protein hydrolysates which are known to be absorbed at a higher rate in normal adults (49) because the former are well defined with a flexible and reproducible composition and can therefore be administered in a more ideal composition corresponding to the known requirements of infants Such diets also eliminate the theoretical risk of abnormal peptide transfer across the small intestinal mucosa in malnutrition

MATERIAL

Patients Data are given in Table 1 The children came from the very low socio-economic group described earlier (35) Most children were anaemic with haemoglobin ranging from 30 to 100 g/l They all showed the classical appearance of marasmus with severe wasting of muscle and subcutaneous tissue and less than 60% of expected weight according to Boston standards or third degree malnutrition (22) The children were chosen for this study consecutively among those seeking admission with third degree malnutrition *and* protracted diarrhoea (four or more loose stools per day for more than 2 weeks) as this complication was associated with the worst prognosis and required the longest period for traditional rehabilitation in a previous local survey

Most of the children were alert their mental condition contrasted with their serious wasting Wrinkling of the conjunctivae (vitamin A deficiency) angular cheilosis and magenta tongue (vitamin B complex deficiency) were seen frequently Six of the infants were after selection for study found to suffer from tubercular pneumonia Streptomycin and INH treatment was in these cases started during the initial dietary rehabilitation (4) The mothers were admitted with the children They all lacked milk for their babies due to maternal disease malnutrition or another pregnancy

METHOD

A screening was made in all cases involving stool examination for eggs and parasites malaria smear and chest X ray Serum electrolytes and bicarbonate were determined before and during the initial i v rehydration

Diet Mean 2 2 days after admission after complete rehydration Vivonex 100® Pfrimmer Co Erlangen was introduced by mouth in amounts of 200 kcal (837 kJ) × kg^{-1} × day^{-1} = 4 5 g amino acids × kg^{-1} × day^{-1} After mean 5 8 days when the infants were completely on peroral Vivonex® feeding a loading test was performed using Vivonex® corresponding to 1 2 g amino acids × kg^{-1} body weight

Vivonex® was chosen because it is packed in sealed packages as a water soluble powder has a good storage stability and provides maximum safety when handled under primitive conditions These advantages were felt to outweigh the fact that the diet is not intended specifically for infants its amino acid composition differing from the known requirements of infants (28) and from that of human milk (Fig 1) The fat content is very low which limits its use for longer trials The carbohydrate content is mainly in the form of glucose and the osmolality is very high Sodium and chloride are much higher while vitamin D iron and zinc concentrations are lower than those required by infants

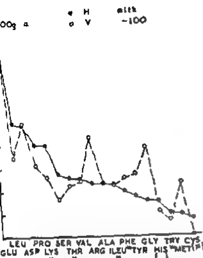


Fig 1 The amino acid composition of Vivonex® as compared to that of human milk (47) Essential amino acids are indicated by an asterix

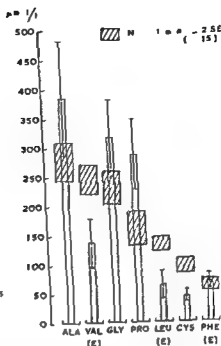


Fig 2 The free amino acid levels of plasma after introduction of the Vivonex® diet Normal levels are those of Stegink & Baker using the same lithium buffer system as in the present investigation (9) Mean ± 2 S E are given for those amino acids whose plasma levels change characteristically in malnutrition

Analytical techniques Cubital vein blood was collected in heparinized tubes the blood was immediately centrifuged the plasma withdrawn and stored in −20°C until transported in the frozen state by a member of the group in Sweden for analysis The plasma was deproteinized with crystalline sulpho-salicylic acid The supernatant was stored in −74 C until analyzed for free amino acid levels on a Beckman 120 B automatic analyzer (52) with the sodium buffer system described earlier (34) or on a Bio-Cal 200 (Munchen) analyzer with a lithium buffer system (9) Human growth hormone and somatomedin levels were assessed after pooling of remaining aliquots from the initial sampling after rehydration (26)

RESULTS

By trying during the period of introduction the different tastes available it was possible to attain a full intake of the diet in all the cases All the infants improved and showed a considerable weight increase (Table 1) There was a tendency to develop a transient phase of looser stools on day 2 and 3 The mothers cooperation was highly satisfactory during the study all of the infants stayed the full period the last case for another week to finish the supply and four reported further improvement later

The original plasma aminogram was characteristic of severe energy deficiency with general hyperaminoacidaemia a slight but significant hyperphenylalaninaemia (80±9 μmol/l) and hyperprolinaemia (353±110 μmol/l) The hyperprolinaemia was sometimes excessive Some of the proline levels were higher than those seen by the authors in any pathological condition (up to 800 μmol/l)

The free amino acid levels of plasma changed during the introduction of the amino acid diet to one characteristic of protein deficiency and excess of carbohydrate with very low branch chained amino acid and cystine levels and marginally high glycine alanine and proline levels (Fig 2) The peroral loads

Table 2 *The elemental diet*

	Vivonex—100®	
Carbohydrate J%	(Glucose)	91
L amino acids J%	(Fig 1)	8
Fat J%	(Safflower oil)	1
Kcal/liter (kJ/liter)	1000 (4184)	
Osmolality mOsm l/liter	840	

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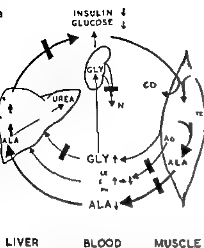
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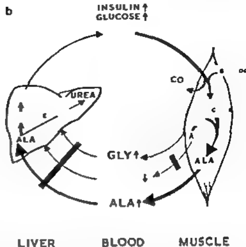
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LIVER BLOOD MUSCLE LIVER BLOOD MUSCLE

Fig 3 Postulated interactions between plasma glucose amino acid and insulin levels in starvation (*a*) and in excess of carbohydrate (*b*) The characteristic changes in different nutritional states (Tables 3 and 4) (17 31 38) are projected onto the glucose—alanine cycle (19 ?1 37) whereby alanine is synthesized in muscle by trans amination of glucose-derived pyruvate Alanine will ac cumulate in arterial blood if the peripheral release exceeds the rate of hepatic uptake (19)

alanine and mainly *glycine* in the kidneys (18) caused by a considerable decrease in the muscle release of alanine but an unchanged glycine release A linear correlation between arterial alanine and pyruvate concentrations has been found during studies of the arterio venous differences of plasma across muscle tissue (19) Insulin secretion as a regulator of hepatic gluconeogenesis for which alanine is the main substrate (18) is indicated in Fig 3 The high sensitivity of the liver to infusion of glucose in normal man (20) could be a conse quence of glucose induced insulin secretion with high endogenous levels of the portal vein as compared to the insulin levels of peripheral blood The correlation between insulin and alanine levels has been verified in a study of nutritional hypoalbuminaemia (36)

The *branch chained* amino acid levels of plasma seem to be especially sensitive to in sulin (17) The initial rise in the branch chained amino acid levels of plasma during ex perimental starvation can be explained by the instant drop in insulin levels (2 31 38) as the plasma valine leucine and isoleucine levels have an indirect relationship with insulin le vels and insulin administration leads to a preferential lowering of these amino acid levels of plasma (16) The subsequent de crease of the plasma levels of these amino acids could be an effect of a decrease in the exogenous supply of these essential amino acids during starvation

The general *hyperaminoacidaemia* seen in severe energy or protein deprivation can be explained by the catabolism induced by the corticosteroid release seen in late stages of starvation (44 60) which thus provides ala nine for hepatic gluconeogenesis from muscle and essential amino acids to specific life sav ing protein synthesis (5) Hyperalaninaemia is a characteristic of acute and chronic gluco-corticoid excess Plasma alanine levels also seem to be correlated to plasma glucagon levels which may explain the augmented ef fect *in vivo* of hypercorticosteroidism on he patic glucose production (60)

DISCUSSION

The pretreatment phase The sometimes ex cessive *hyperprolinaemia* seen in the maras mic infants of the present investigation has not been described earlier It could be a result of increased collagen breakdown in the cata

Table 3. *The post absorptive free amino acid levels of plasma as affected by different types of malnutrition*

Type	Increased levels (or unchanged levels during a general decline)	Decreased levels
1. Experimental energy undernutrition (starvation) *Adults* (2, 16)	Glycine	Valine Leucine Isoleucine* Alanine α NH_2-N
2. Experimental protein undernutrition *Children* (6, 51)	Glycine Alanine Proline	Valine Leucine Isoleucine Tyrosine Lysine
Adults (2, 55)	Glycine Alanine	Valine
3. Protein deficiency syndrome (kwashiorkor) of *children* (6, 14, 29, 46, 58)	Glycine Alanine Proline Histidine Phenylalanine Lysine	Valine Leucine Isoleucine Tyrosine Arginine α NH_2 N
4. Experimental protein overnutrition *Children* (51)	Valine Leucine Isoleucine Proline	Glycine
5. Experimental carbohydrate overnutrition *Adults* (20)	Glycine Alanine	Valine Leucine Isoleucine Tyrosine Phenylalanine

* Decrease after a temporary increase.

showed only a significant rise of methionine at 2 hours during the Vivonex® load. There was a general tendency towards low initial levels and a fast return to even lower levels during the amino acid loads.

The human growth hormone levels were slightly elevated (mean 7.5 ng/ml). Somatomedin was not detectable in pooled samples from these marasmic infants.

REVIEW

The change in the postabsorptive levels of plasma free amino acids seen in malnutrition runs through many phases depending on the severity and the ratio of protein/energy deprivation. A review of the extensive literature on the subject is given in Tables 3 and 4.

The investigations of the arteriovenous differences of plasma amino acids across muscle tissue (19), liver (11, 16, 20) and kidney (41) in different nutritional states, and during work load, offer a tentative explanation of the consistent and characteristic findings in all age groups. The low glucose and insulin levels during experimental and clinical starvation (7, 16, 31, 38), the low alanine, branch chained, tyrosine, and phenylalanine levels and the high glycine levels (Tables 3 and 4) are projected onto the "glucose alanine cycle" (19, 21, 37) in Fig. 3*a*. The high alanine and low branch chained amino acid levels in experimental low protein supply, or in clinical protein deficiency, or high carbohydrate supply (Table 3 and 4) are projected onto the same hypothetical cycle (20) in Fig. 3*b*.

Alanine seems to be the key protein derived gluconeogenetic precursor (21). During starvation, there seems to be a shift from gluconeogenesis from alanine in the liver to that of

Table 4. *Characteristics of the post absorptive free amino acid levels of plasma in different nutritional states according to the literature (Table 3)*

↑=Increased plasma levels, or unchanged levels during a general decline. ↓=Decreased levels.

1	(*a*) Subclinical protein deficiency (nitrogen lack)	VAL ↓	LEU ↓	ILEU ↓	TYR ↓
	(*b*) *Severe* protein deficiency	GLY ↑ HIS ↑	*ALA* ↑ PHE ↑	 LYS ↑	
2	(*a*) Energy deficiency, early (in addition to, or differences from 1.)	*ALA* ↓	α NH_2N ↓		
	(*b*) *Severe* energy deficiency	All amino acids ↑			
3	Protein malnutrition in children (N lack or excess)	PRO ↑			

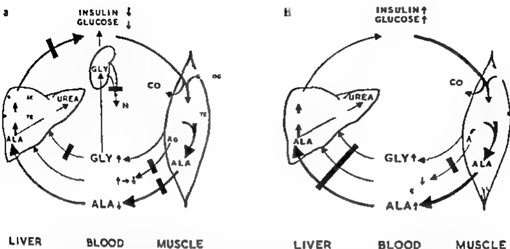


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The general *hyperaminoacidaemia* seen in severe energy or protein deprivation can be explained by the catabolism induced by the corticosteroid release seen in late stages of starvation (44 60) which thus provides alanine for hepatic gluconeogenesis from muscle and essential amino acids to specific life saving protein synthesis (5) Hyperalaninaemia is a characteristic of acute and chronic glucocorticoid excess Plasma alanine levels also seem to be correlated to plasma glucagon levels which may explain the augmented effect *in vivo* of hypercorticosteroidism on hepatic glucose production (60)

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bolic state of the severely starved infants especially as the proline excretion from muscle is known to be relatively high but the liver extraction and glucose production from proline is comparatively low (21) Hydroxyproline excretion in the urine is correlated to growth and has already been used in the assessment of nutritional status (59)

The deranged plasma homeostasis before treatment may be a result of the beneficial hormonal adaptation to low nutrient supply as suggested in the review above but may also in itself have important pathological consequences Raised alanine levels seem to be directly associated with increased liver uptake and presumably gluconeogenesis (18) but the accompanying high proline levels seen in this study may have neurotoxic effects Proline in addition to glutamic and aspartic acid has a unique sodium dependent high efficiency transport system in cerebral cortical nerve terminals and is selectively released by potassium depolarization (50) This makes it into an inhibitory transmitter candidate of the cerebral cortex parallel to the function of glycine in the spinal cord From studies of cerebral exchange of nutrients in infants (48) it seems that the increase of the arterial level of an amino acid increases the cerebral arteriovenous difference of that amino acid The risk of competition for transfer across the blood brain barrier (40) must be considered

Human growth hormone levels of plasma are known to be high or normal in severe nutritional growth retardation (36) The factor induced by HGH *somatomedin* (26) was not detectable in the present cases The factor is stable for years in frozen plasma Low somatomedin levels of plasma have been found in kwashiorkor (24) The pronounced effects of short term fasting and refeeding on somatomedin activity have been described in rats (42) Further studies should aim to evaluate whether there is a mechanism whereby growth may be inhibited through decreased synthesis of somatomedin during the process of adaptation to protein deprivation

The peroral load The only significant change being an increase of plasma methionine after the Vivonex® load is not surprising as the methionine concentration of this diet is relatively high (Fig 1) and methionine is the most rapidly absorbed amino acid into raised plasma levels after equimolar intestinal amino acid perfusion in man (1)

The rise of plasma amino acids after a protein rich meal seems to stem from the amino acids of exogenous source (3) in normal subjects However peroral loads do not mirror intestinal absorption alone and the metabolic situation of infants suffering from nutritional growth retardation is more complex than that of mere substrate depletion The hypoglycaemia hypothermia and high mortality seen in the traditional rehabilitation of severe undernutrition (57) and the change in body composition (39) suggest a complex metabolic adaptation to low nutrient supply Considering the short half time of free amino acids in plasma under normal condition and the high nitrogen turnover protein catabolism and protein synthesis in undernutrition (43) the results of the peroral loads are difficult to interpret

Because of the facts mentioned above the present study gave more attention to the change of the postabsorptive levels 4 hours after the test load and after an overnight fast Low pre load levels and a fast return to initial levels in addition to the general picture of feasting or carbohydrate excess in the use of Vivonex® suggests a rapid absorption an insulin release and a change from a catabolic to an anabolic state This may be explained by the very high content of glucose in Vivonex® The composition of the elemental diet may be critical for the optimal absorption of amino acids and the quick normalization of the plasma amino acid levels

This investigation (Figs 1 and 2) supports the assumption that cystine is an essential amino acid not only to the newborn human infant (54) but also to chronically starved infants as treatment with a diet devoid of cystine

ed to very low plasma levels of this particular amino acid

SUMMARY AND CONCLUSIONS

Severely marasmic infants from a very low socio-economic group of a developing coun try suffering from chronic diarrhoea were initially rehabilitated with a commercially available amino acid—glucose diet The re markably good results with no early deaths and 100% improved [illegible] probably due to the meticulous initial water and electrolytical treatment rather than the diet *per se* If an elemental diet is to be used for a prolonged treatment of malnourished infants it has to be considerably modified

It seem from the investigation of the change in the plasma aminogram that the infants did not absorb sufficient free amino acids to keep pace with the carbohydrate supply This is probably due to the fact that the elemental diet contains relatively large amounts of glu cose and that free amino acids are less effi ciently absorbed than peptides However the perorally administered amino acids could be mainly or partially used for necessary local mucosal repair during the initial phase of die tary rehabilitation followed by a later in creased absorption rate

ACKNOWLEDGEMENTS

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The peroral load The only significant change being an increase of plasma methionine after the Vivonex® load is not surprising as the methionine concentration of this diet is relatively high (Fig 1) and methionine is the most rapidly absorbed amino acid into raised plasma levels after equimolar intestinal amino acid perfusion in man (1)

The rise of plasma amino acids after a protein rich meal seems to stem from the amino acids of exogenous source (3) in normal subjects However peroral loads do not mirror intestinal absorption alone and the metabolic situation of infants suffering from nutritional growth retardation is more complex than that of mere substrate depletion The hypoglycaemia hypothermia and high mortality seen in the traditional rehabilitation of severe undernutrition (57) and the change in body composition (39) suggest a complex metabolic adaptation to low nutrient supply Considering the short half time of free amino acids in plasma under normal condition and the high nitrogen turnover protein catabolism and protein synthesis in undernutrition (43) the results of the peroral loads are difficult to interpret

Because of the facts mentioned above the present study gave more attention to the change of the postabsorptive levels 4 hours after the test load and after an overnight fast Low pre load levels and a fast return to initial levels in addition to the general picture of feasting or carbohydrate excess in the use of Vivonex® suggests a rapid absorption an insulin release and a change from a catabolic to an anabolic state This may be explained by the very high content of glucose in Vivonex® The composition of the elemental diet may be critical for the optimal absorption of amino acids and the quick normalization of the plasma amino acid levels

This investigation (Figs 1 and 2) supports the assumption that cystine is an essential amino acid not only to the newborn human infant (54) but also to chronically starved infants as treatment with a diet devoid of cystine

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ACUTE HEMOLYTIC ANEMIA RELATED TO DIPHTHERIA PERTUSSIS TETANUS VACCINATION

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ABSTRACT Haneberg B Matre R Winsnes R Dalen A Vogt H and Finne P H (Department of Paediatrics Broegelmann Research Laboratory for Microbiology and Department of Microbiology University of Bergen Bergen and Vaccine Department National Institute of Public Health Oslo Norway) Acta Paediatr Scand 67 345 1978 —Three infants developed severe hemolytic anemia following the second or third diphtheria pertussis tetanus vaccination Direct antiglobulin tests were positive and the infant most severely affected also had reduced serum complement levels indicating an immunological mechanism for the hemolysis The presence of IgM on the erythrocytes from 2 of the infants could be demonstrated by antiglobulin tests or immunization experiments Heat eluates of the erythrocytes from one of the infants contained antibodies to tetanus and diphtheria toxoids as well as to *Bordetella pertussis* suggesting that these antibodies were antigenically bound to the erythrocytes Virus antibodies or isoagglutinins present in the serum were not found in the eluate No antibodies against the vaccine components could be demonstrated in eluates of erythrocytes from control subjects *In vitro* experiments showed that tetanus and diphtheria toxoids were easily bound to human erythrocytes This finding could help explain the pathogenesis of the autohemolysis

KEY WORDS Hemolytic anemia DPT vaccination

Acute hemolytic anemia can occur at any age even in early infancy (2 6) Both infections and drugs have been implicated as causative factors (11 22) Tetanus toxoid can provoke local as well as generalized side effects (5 21) probably due to immune mechanisms (4) Local reactions are also seen with the diphtheria vaccine (16) The pertussis vaccine is regarded to be responsible for severe neurological reactions (19) The present paper concerns three patients who developed acute hemolytic anemia following the second or third dose of diphtheria pertussis tetanus (DPT) vaccine The immunological factors underlying the hemolytic disease have been searched for

CASE REPORTS

Case 1 a boy was born Oct 1973 as the fifth child of healthy unrelated parents At 3 months of age he received the first dose of DPT vaccine produced by the National Institute of Public Health The vaccine was adsorbed onto aluminium phosphate and it fulfilled the WHO requirements (14 15) The second subcutaneous injection was given 4 weeks later Four days thereafter he was admitted to hospital because of severe anemia

On admission he was pale and jaundiced His spleen was palpable no other abnormality was found on physical examination Hemoglobin concentration was 49 g/l The erythrocyte number was $1\ 95\times10^{12}$/l the reticulocyte number fraction was 1×10^{-3} Further hemoglobin and reticulocyte values are given in Fig 1 The leucocyte differential and thrombocyte counts were normal The maximum serum bilirubin level was 137 mmol/l Haptoglobin was not detectable until after remission The initial serum carbamide concentration was 9 8 mmol/l but creatinine concentration was normal Serum IgA IgG IgM and IgE concentrations were within normal ranges for the boy s age The concentrations of the complement factors C3 and C4 gradually fell from normal values to the lowest values 68 and III mg/l respectively on day 10 after the second vaccination Urine cultures were negative for viruses and bacteria as were also serologic tests for mycoplasma and rubella measles influenza A and B adeno- and cytomegaloviruses During the first few days the urine was reddish and positive for blood Bone marrow smears showed erythroid hyperplasia

Table 2 *Antibodies in serum and in eluate of erythrocytes from an infant with acute hemolytic anemia*

Antibodies against diphtheria and tetanus toxoids *Bordetella pertussis* and against polio- and cytomegalovirus as well as isoantigens A and B were tested for in serum and in eluate of washed erythrocytes from case 3 who had blood group O

	IU per ml		Titers						
	Diphtheria	Tetanus	Pertussis	Polio (type 1 2 3)			CMV	Isoantigen A	B
Serum	>10	10	10	64	32	16	64	32	32
Eluate	0 003	0 00	<5 (trace)	<16	<16	<16	<1	<1	<1

restricted to an antiserum to IgM IgM therefore was probably coating the boy's erythrocytes during the acute stage of the disease

The monovalent aluminium phosphate adsorbed diphtheria and tetanus vaccines each agglutinated erythrocytes from cases 2 and 3 and from healthy individuals both vaccinated and unvaccinated (Table 1) Non adsorbed vaccines were not tested The erythrocytes were diluted to 0 25% in phosphate buffered saline (PBS) pH 7 2 with 1% bovine serum albumin and the agglutinations were read under microscope The tests were made at a time when the patients were healthy and had negative antiglobulin tests

The two patients sera as well as sera from vaccinated healthy controls agglutinated their own erythrocytes which were sensitized with either diphtheria or tetanus vaccine the titers were 8–16 and 4–256 *respectively* For sensitizations of the erythrocytes were used 0 1 agglutinating units which were defined as the amount of vaccines that corresponded to the titer One ml of the sensitized erythrocytes was washed 3 times in 20 ml PBS before serum was added *These results* give evidence that the vaccines and notably the tetanus component attached to human erythrocytes

Poliovirus type 2 diluted to 10^6 infectious units per ml and cytomegalovirusantigen strain AD 169 cultured on human lung fibroblasts diluted to the concentration used for routine complement fixation reaction *did not* agglutinate the human erythrocytes Complement dependent lysis using various dilutions of guinea pig serum could not be evoked with any of the sensitized erythrocytes in the presence or absence of specific antibodies

Antibody elution Erythrocytes from case 3 and from 3 healthy vaccinated controls were washed 4 times in PBS each time using 20 ml PBS to 1 ml erythrocytes which were separated by centrifugation at 1000 g for 10 min Eluates of the washed erythrocytes from each of these individuals were made by incubating 1 ml of packed erythrocytes in 1 ml isotonic saline at 56 C (20 min) The erythrocytes were then separated at that temperature by centrifugation as before The supernatant called the eluate was tested for antibody activity

Antibody assays The levels of tetanus antitoxin in sera and eluates were determined by injections in mice of a toxin test dose mixed



Fig 2 Immunoelectrophoresis of normal human serum (wel) against rabbit antiserum to the washed erythrocytes from case 1 a 4-month-old boy with hemolytic disease (upper trough) and against that same antiserum absorbed to remove anti light-chain activity (lower trough) *The precipitation lines are indicated by arrows*

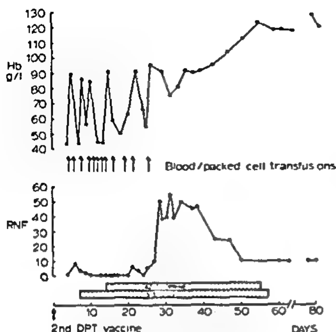


Fig 1 Hemoglobin (Hb) values and reticulocyte number fractions (RNF)×10⁻³ during the hemolytic disease of a 4 month old boy The therapy is indicated

The boy was treated with prednisone and azathioprine for about 6 weeks in addition to repeated transfusions of whole blood and packed red cells (Fig 1) After 8 weeks he was discharged in complete remission On later controls he was healthy

Case 2 a girl was born Nov 19 1973 after uneventful pregnancy as the fourth child of healthy unrelated parents Nearly 7 months old she received the second DPT vaccine and the first trivalent oral poliovaccine Two weeks later she was jaundiced and a reddish discoloration of the urine was noted

On admission to hospital one week thereafter she was pale and the spleen was palpable Hemoglobin concentration was 28 g/l The reticulocyte number fraction amounted to 70×10^{-3} The leucocyte differential and thrombocyte counts were normal Serum bilirubin reached 178 mmol/l The lowest haptoglobin concentration was 1 4 mg/l The serum concentrations of carbamide immunoglobulins and complement factors C3 and C4 were within normal limits as were also the serum levels of alanine aminotransferase alkaline phosphatase and γ glutamyl transferase High antibody titers against influenza B virus was found no other serum virus antibodies were detected Urine cultures were negative for bacteria and viruses Bone marrow smears showed marked erythroid hyperplasia

The girl was treated with prednisone and azathioprine as well as transfusions of whole blood and packed red cells She was discharged in apparent remission after 4 weeks On follow ups she remained healthy despite elevated reticulocyte counts as a sign of continuing hemolysis which lasted for a year After that no abnormalities were noted

Case 3 a boy was born Jan 1 1976 as the first child of healthy unrelated parents He received DP[illegible] vaccines at 3½ 4½ and 5½ months of age and got [illegible] trivalent oral poliovaccine at 9 months He was very p[illegible] for weeks before he was admitted to hospital at the [illegible] of 10½ months

On admission he was pale and the spleen was [illegible] pable Hemoglobin concentration was 54 g/l The [illegible] reticulocyte number fraction was high 1040×10^{-3} T[illegible] leucocyte differential and thrombocyte counts were normal The haptoglobin concentration was low 0 ? mg/l The serum concentrations of immunoglobulins and complement factors C3 and C4 were within normal limits for his age as were the concentrations of carbamide alanine aminotransferase and γ glutamyltransferase Antibodies against cytomegalo- and polioviruses were found in his serum no other virus antibodies were detected Urine culture was negative for viruses Poliovaccinevirus type 2 was demonstrated in his stools Bone marrow smears showed erythroid hyperplasia

This boy was the least affected of the three being treated with blood transfusion alone At later follow ups he was healthy with no signs of hemolysis

Table 1 *Agglutination of human erythrocytes by monovalent diphtheria pertussis and tetanus vaccines*

Erythrocytes from case 2 and case 3 recovered from acute hemolytic anemia and 5 healthy controls were tested

	Titers of the vaccines		
Erythrocytes	Diphtheria	Pertussis	Tetanus
Case 2	64	4	1.8
Case 3	16	<2	64
Control vaccinated	32	<2	1?8
Control unvaccinated	16	<4	256
Control unvaccinated	8	<4	256
Control unvaccinated	32	<4	256
Control unvaccinated	8	<4	256

EXPERIMENTS AND RESULTS

Agglutination tests Agglutinins against the infants own blood groups were not found in their sera

Direct antiglobulin tests using polyvalent antisera to IgA IgG and IgM as well as to complement factors were positive with washed erythrocytes from all cases Erythrocytes from cases 1 and 2 were not tested with antisera specific for any of these factors In case 3 the specificity of this reaction was

ing that the diphtheria and tetanus vaccines had an affinity to human erythrocytes could therefore help explain the initial step in the pathogenesis of the hemolysis

Positive results of antiglobulin tests in all our patients indicate that the hemolysis most likely was the result of an immunological reaction This assumption was strengthened by decreased serum complement levels in the severely affected case 1 during the acute stage of the disease

Antiglobulin tests with antiserum to IgM and immunization of rabbits with human erythrocytes give evidence that IgM was coating the erythrocytes from at least two of our patients This is in accordance with the finding of others that 7 S IgM may have immunological activity (18) and that such incomplete IgM antibodies may be responsible for hemolytic reactions (3)

The demonstration of antibodies against the 3 components of the DPT vaccine in the eluate of the erythrocytes from case 3 shows that these antibodies were bound to the erythrocytes Thus the hemolytic disease can be explained by the action of antibodies against the vaccine components that were already attached to the surface of the erythrocytes

Serious complications from the vaccine such as in our three cases must be rare since most individuals in Norway have received three injections of diphtheria pertussis tetanus vaccine during infancy and booster injections of this or diphtheria tetanus vaccine at a later age without such complications having been reported Serious reactions may however have been overlooked and less dramatic reactions as in case 3 are not evident until a considerable time has elapsed making a relationship with vaccination less obvious

At present investigations on the immune status after immunization with DPT vaccine according to the schedules recommended by the Norwegian National Health Authorities are performed by the National Institute of Public Health Since a correlation between antibody response and untoward reactions seems to exist the immunization schedules will be reevaluated considering the necessary antibody level for protection

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with dilutions of samples (7) In addition serum samples were assayed by hemagglutination (17) Rabbit skin test was used for determination of diphtheria antitoxin (8) Antibodies against *Bordetella pertussis* were evaluated by complement fixation technique (12) The results (Table 2) showing antibodies in the eluate of erythrocytes from case 3 against each of the components of the diphtheria pertussis tetanus vaccine suggest that these antibodies were antigenically bound to the erythrocytes On the other hand no other antibodies present in his serum were found in this eluate and no antibodies were found in the eluates of the control erythrocytes even though their serum antibody levels corresponded to or were higher than the serum antibodies of case 3

Immunization experiments Antisera to the erythrocytes from cases 1 and 3 as well as from three healthy vaccinated controls were obtained by immunization of 5 rabbits One ml washed and packed erythrocytes was mixed with equal amounts of complete Freund s adjuvant and given intramuscularly After 3 weeks the rabbits received a second injection of erythrocytes stored with incomplete Freund s adjuvant at −20°C One week thereafter antisera were obtained by bleeding the rabbits

By immunoelectrophoresis of normal human serum against the rabbit antisera to the erythrocytes of cases 1 and 3 precipitin lines representing both IgG and IgM were detected (Fig 2) However, after absorption of the antisera by sheep erythrocytes sensitized with human serum IgM (10) both precipitin lines were removed After absorption by rabbit erythrocytes sensitized with either human serum IgG (10) or salivary IgA (20) the IgM line only persisted Thus the IgM line represented activity against μ heavy chains while the IgG precipitin line seemed to represent anti light chain activity No antibodies against human immunoglobulins were found in sera from the rabbits immunized with the control erythrocytes

A precipitate in the α region was demonstrated on immunoelectrophoresis of normal human serum against the antisera to the erythrocytes of cases 1 and 3 (Fig 2) With use of antisera (Behringwerke AG Marburg Lahn Germany) this α precipitate could not be identified to represent any of the following serum proteins albumin $\alpha_1$acid glycoprotein $\alpha_1$antitrypsin $\alpha_1$antichymotrypsin β_1C/β_1A globulin (C3) and β_1E globulin (C4)

DISCUSSION

The severe anemia low serum haptoglobin concentrations and erythroid hyperplasia in the bone marrow of our patients gave evidence of hemolysis In the two infants most severely affected the transfused erythrocytes were also rapidly destroyed Despite the signs of marked erythropoiesis the number fractions of circulating reticulocytes of case 1 remained low until early clinical remission This indicates lysis at an early stage of erythrocyte development and is of grave prognostic significance (1)

The appearance of hemolysis shortly after the second DPT vaccination in cases 1 and 2 implicates the vaccine as a possible causative agent Both immediate Arthus type and delayed immune reactions are frequently seen by skin testing after previous tetanus immunization (4) and seem related to the frequency of booster injections (9) However the reactions have not been life threatening (21) Autoimmune hemolytic reaction has previously been reported in one case following DPT vaccination but the etiologic relationship was not established (23) Hemolytic uremic syndrome have also been reported with close time relationship to DPT vaccination but without evidence of an immune reaction (13)

The drugs involved in hemolytic reactions may act as a hapten with affinity to the red cell membrane (11) This is seen during treatment with large doses of penicillin and antibodies act against the drug adsorbed to the cell surface The results of our experiments show

ing that the diphtheria and tetanus vaccines had an affinity to human erythrocytes could therefore help explain the initial step in the pathogenesis of the hemolysis

Positive results of antiglobulin tests in all our patients indicate that the hemolysis most likely was the result of an immunological reaction This assumption was strengthened by decreased serum complement levels in the severely affected case 1 during the acute stage of the disease

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URINARY CYCLIC AMP IN INFANTS ADMITTED TO A NEONATAL INTENSIVE CARE UNIT

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ABSTRACT Aksnes L Søvik [redacted] Finne P H and Opshaug [redacted] (Department of Paediatrics University of Bergen Norway) Urinary cyclic AMP in infants admitted [redacted] a neonatal intensive care unit Acta Paediatr Scand 67 351 1978 —The urinary excretion of cyclic AMP was studied during the first 3 days of life in 46 randomly selected infants admitted [redacted] a neonatal intensive care unit The data were compared with those of normal newborn infants Urinary cyclic AMP concentrations were significantly correlated with gestational age (all patients) and with birth weight (all patients except infants of diabetic mothers (IDMs)) The urinary cyclic AMP/creatinine ratio increased from day 1 to day 3 in normal newborns and in IDMs and tended [redacted] increase also in small for-gestational age (SGA) low birth weight (LBW) and sick term infants although the changes in the latter groups were not statistically significant. Four infants studied with parallel determinations showed increased cyclic AMP/creatinine ratio from day 1 to day 3 both in plasma and urine All urinary cyclic AMP/creatinine ratios were lower than the corresponding ratios found in plasma In LBW infants there was an inverse relationship between urinary cyclic AMP and serum calcium In IDMs a positive correlation was observed between urinary cyclic AMP and blood glucose concentration In conclusion the excretion of cyclic AMP in sick newborn infants [redacted] influenced by the following factors gestational age postnatal age birth weight and derangements of serum calcium and blood glucose concentrations

KEY WORDS Newborn hypocalcaemia diabetes mellitus cyclic AMP

Adenosine 3 5 monophosphate (cyclic AMP) which is the intracellular mediator of a large number of hormonal effects (5) is excreted [redacted] the urine in increasing amounts during the first days of life (12) Studies in adult man have shown that cyclic AMP is partly cleared from plasma by glomerular filtration approximately one third being added in the kidneys by tubular secretion (3) Whereas the nephrogenous cyclic AMP [redacted] mainly determined by parathyroid hormone (PTH) (6) extra renal cyclic AMP is influenced by glucagon catecholamines and other hormones (4 5)

The aim of the present study was to examine which factors might influence the urinary excretion of cyclic AMP in sick newborn infants and in particular to evaluate the effects of hypocalcaemia and hypoglycaemia We therefore studied the urinary excretion of this nucleotide in a group of randomly selected infants admitted to a neonatal intensive care unit Values obtained in small for gestational age (SGA) low birth weight (LBW) and sick term infants and infants of diabetic mothers (IDMs) were compared with those observed in normal newborn babies

MATERIALS AND METHODS

Patients and controls

Forty six infants admitted [redacted] the neonatal unit were studied Their birth weights and gestational age are shown in Table 1 The material included [redacted] LBW infants 17 IDMs 8 SGA and 8 sick term infants Gestational age was determined by history and clinical examination The SGA infants were all below the 2 5 percentile on the local intrauterine growth chart The sick term infants were observed for conditions such as convulsions asphyxia and high serum bilirubin The normal newborn infants serving as controls were studied during their stay in the maternity ward

Table 1 *Clinical data of patients studied*

Group of patients	No of patients	Birth weight (g)	Gestational age (weeks)
IDM	12	3595±739	37 7±1 9
SGA	8	2142±393	38 0±2 2
LBW	18	1952±538	33 9±2 1
Term infants	8	3426±437	39 4±1 3

Mean ±S D

Urine and blood sampling

Urine samples were as a rule obtained in the morning and stored at −20°C until analyzed If possible samples were obtained from each patient during the first 24 hours (day 1) from 24–48 hours (day 2) and from 49–72 hours (day 3) In some cases capillary blood samples were obtained as well The blood was drawn into EDTA and the plasma was separated as soon as possible and stored at −20°C until analyzed

Analytical methods

Cyclic AMP was determined by a competitive protein binding assay based on a binding protein preparation isolated from bovine skeletal muscle (1) Urinary cyclic AMP was determined in diluted samples (1 200 in distilled water) Plasma samples were analyzed directly Creatinine in urine was determined by the Jaffe reaction as described by Natelson (15) Serum creatinine was determined by the resin adsorption micromethod described by Stoten (16) Blood glucose was analyzed by the orthotoluidine method (11) Serum glucose was analyzed by the orthotoluidine method (11) Serum calcium was determined by conventional technique

Hypocalcaemia was defined as serum calcium below 8 0 mg/100 ml (=2 00 mmol/l) in full term and below 7.5 mg/100 ml (=1 88 mmol/l) in preterm infants

Statistics

The level of significance was calculated using Student's *t* test for two means or the paired *t* test as judged appropriate The regression line was calculated by the method of least squares

RESULTS

Fig 1 shows that when the mean urinary concentration of cyclic AMP during the first 3 days of life was related to gestational age there was a highly significant correlation ($p<0\,001$) Similarly there was a statistically significant correlation between cyclic AMP concentration and birth weight ($p<0\,02$) As shown in Fig 2 the values of IDMs which were excluded from the calculation of the correlation coefficient fell below the regression line except for one point

Table 2 gives the urinary concentrations of cyclic AMP and creatinine for the different groups of patients on day 1 2 and 3 Although the values varied considerably from patient to patient it appeared that creatinine and to some extent cyclic AMP showed the highest concentrations on day 1

The urinary cyclic AMP/creatinine ratio increased almost 2 fold from day 1 to day 3 in normal newborn infants A slightly smaller in

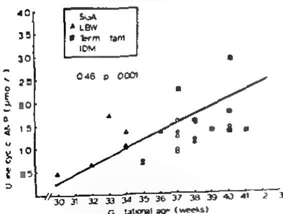


Fig 1 Relation of urine cyclic AMP concentration to gestational age For each patient the mean value of the urinary cyclic AMP concentration for the first 3 days of life was calculated and related to gestational age Each point represents one patient

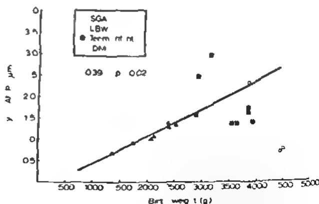


Fig 2 Relation of urine cyclic AMP concentration to birth weight Cyclic AMP concentration calculated as mentioned in legend to Fig 1 was related to birth weight Each point represents one patient

Table 2 *Urine concentrations of cyclic AMP and creatinine in infants studied*

Group of infants	Creatinine (mg/100 ml)			Cyclic AMP (nmol/l)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
IDM	41±11 (9)	31±6 (11)	34±7 (10)	1261±194 (9)	1178±161 (11)	1424±313 (10)
SGA	60±24 (7)	41±10 (7)	34±10 (5)	1843±679 (7)	1434±336 (7)	1100±?97 (4)
LBW	53±14 (11)	?0±2 (14)	?5±4 (15)	1554±352 (10)	871±136 (14)	1058±129 (15)
Term infants	80±?? (7)	53±13 (8)	49±20 (4)	1797±3?6 (7)	1855±453 (8)	1783±318 (4)
Normal newborns	59±13 (12)	66±15 (6)	41±18 (4)	?033±358 (1?)	2843±587 (6)	2454±90? (4)

Mean values ±S E Number of infants in parentheses

Table 3 *Serum calcium in patients studied*

The left column shows the mean value of the lowest serum calcium concentration observed in each patient To the right are shown the mean serum calcium values in the different groups of patients on the first 3 days of life

Group of patients	Serum calcium (mmol/l)			
	Mean of lowest values	Mean day 1	Mean day 2	Mean day 3
IDM	? 04±0 07 (12)	*2 44±0 05 (1?)	*? 06±0 07 (9)	2 15±0 07 (8)
SGA	? 00±0 [illegible] (8)	2 36±0 09 (8)	2 25±0 08 (8)	2 15±0 11 (8)
LBW	1 91±0 05 (18)	*2 28±0 04 (18)	*2 03±0 06 (18)	2 11±0 07 (17)
Term infants	? 16±0 03 (8)	2 47±0 05 (8)	? 20±0 06 (7)	2 33±0 11 (6)

Mean values ±S [illegible] number of patients in parentheses
$p<0\ 001$

crease was observed in IDMs The same trend with a gradual increase from day 1 to day 3 was observed also in SGA LBW and term infants but in these groups the increment was not statistically significant The 24-hour output of cyclic AMP studied in 2 cases (1 LBW 1 SGA) increased from 0 041 on day 1 to 0 070 on day 3 (µmol/24 hours)

Table 3 shows serum calcium values for IDMs SGA LBW and term infants during the first 3 days of life The lowest serum calcium concentrations were observed in LBW infants In IDMs and LBW infants there was a highly significant decrease when mean values on Day 1 and Day 2 were compared This decrease was not statistically significant in SGA infants

The lowest blood glucose concentrations

Table 4 *Blood glucose concentrations in infants studied*

The left column shows the mean value of the lowest blood glucose concentration observed in each patient To the right are shown the mean blood glu ose values in the groups of patients on the first 3 days of life

Group of patients	Blood glucose concentration (mmol/l)			
	Mean of lowest values	Mean day 1	Mean day 2	Mean day 3
IDM	1 63±0 1 (1?)	*? 69±0 1? (1)	*3 21±0 0 (10)	3 26±0 36 (7)
SGA	1 63±0 37 (8)	2 67±0 39 (8)	2 87±0 4 (8)	2 19±0 .6 (4)
LBW	8 ±0 18 (18)	3 36±0 [illegible] (18)	4 13±0 3? (11)	
Term infants	55±0 35 (8)	3 41±0 49 (8)	3 28±0 17 (5)	2 97 (2)

Mean values ±S E number of patients in parentheses
* $p<0\ 05$
$p<0\ 0?$

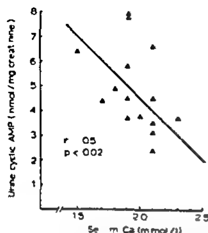


Fig 3 Relation of urine cyclic AMP/creatinine ratio to serum calcium in low birth weight infants For each patient the mean urinary cyclic AMP/creatinine ratio for the first 3 days of life was calculated and related to the lowest serum calcium value observed on these days Each point represents one patient

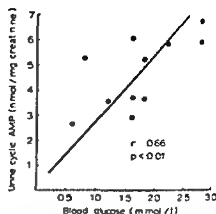


Fig 4 Relation of urine cyclic AMP/creatinine ratio to blood glucose in infants of diabetic mothers Urinary cyclic AMP/creatinine ratio calculated as mentioned in legend to Fig 3 was related to the lowest blood glucose concentration observed on the first 3 days of life Each point represents one patient

were observed in IDMs and SGA infants (Table 4) The blood glucose concentration increased from day 1 to day 2 in IDMs and LBW infants whereas no change was observed in SGA infants In the latter group the blood glucose tended to decrease towards day 3

In Fig 3 the mean urinary cyclic AMP/creatinine ratio for the first 3 days of life is related to the lowest serum calcium concentration of each patient (LBW) Low cyclic AMP excretion was associated with high serum calcium and vice versa

In Fig 4 a similar plot is shown for blood glucose values and mean urinary cyclic AMP/creatinine ratios in IDMs In these infants there was a statistically significant positive correlation between cyclic AMP excretion and blood glucose concentration

Table 5 shows the concentration of cyclic AMP in plasma and the plasma and urinary cyclic AMP/creatinine ratio on day 1 and day 3 in 4 infants (3 LBW 1 SGA) In two of the patients there was an increase in plasma cyclic AMP concentration from day 1 to day 3 All patients showed increased cyclic AMP/

Table 5 *Plasma cyclic AMP and plasma and urinary cyclic AMP/creatinine ratios on day 1 and day 3*

Three LBW infants (K D K G S N) 1 SGA infant (H P)

Patient studied	Day	Plasma cyclic AMP (nmol/l)	Plasma cyclic AMP/creatinine (nmol/mg)	Urinary cyclic AMP/creatinine (nmol/mg)
K D	1	43 4	7 4	4 1
	3	52 8	8 8	4 4
H P	1	41 0	3 3	2 1
	3	81 0	8 3	3 9
K G	1	31 5	4 0	2 8
	3	30 0	4 9	3 6
S N	1	44 5	4 8	3 0
	3	39 2	5 4	5 1

creatinine ratios in both plasma and urine during the same period All urinary cyclic AMP/creatinine ratios were lower than the corresponding ratios found in plasma

DISCUSSION

In the present work it was observed that the concentration of cyclic AMP in urine of new born infants increased with gestational age It appears therefore that the production of this nucleotide is associated with the process of development during the later part of intra uterine life

The concentration of cyclic AMP was also positively correlated with birth weight Pre viously Ling et al (13) have established a positive correlation between the concentration of cyclic AMP in amniotic fluid and the corre sponding birth weight of the newborn infant It is of interest that in our work the IDMs diverged from the correlation line shown by the other groups of infants studied This supports the view that urinary cyclic AMP is an index of maturation and not related to body weight as such

The pattern of urinary cyclic AMP in the early neonatal period with an increasing cyclic AMP/creatinine ratio from day 1 to day 3 was first demonstrated by Linarelli et al (12) The data of Maxwell & Dahlenburg (14) show an increase of this ratio from day 1 to day 5 in normal fullterm babies In the present work the 4 groups of patients studied and also the normal newborn infants showed essen tially the same pattern during the first 3 days of life with an increasing urinary cyclic AMP/creatinine ratio

In using creatinine as a reference it should be realized that the urinary concentration of this compound tends to decrease during the first days of life (ref 14 and present work) It may therefore be argued that the excretion of creatinine rather than that of cyclic AMP is changed However we feel justified in con cluding that the excretion of cyclic AMP through the kidneys increases in the early neo natal period Thus there were no major changes in the urinary concentration of the nucleotide from day 1 to day 3 and it is well known that the 24 hour urine volume increases considerably during this period Indeed this conclusion was confirmed by direct measure ment of the 24 hour output of cyclic AMP in two cases

Linarelli et al (12) obtained evidence of an increasing tubular responsiveness to PTH during the first days of life and suggested that there was an increased urinary excretion of cyclic AMP due to increasing production in the renal tubules Observations in the present work suggest that there may be additional important changes in the amount of cyclic AMP filtered through the glomeruli Thus there was an increase of the plasma cyclic AMP/creatinine ratio from day 1 to day 3 In fact the increase in urinary cyclic AMP/creatinine ratio may well be explained by the increase in plasma ratio The renal clearance rate of cyclic AMP in adult man was studied by Broadus et al (3) who concluded that the clearance was almost identical to that of creatinine and unaltered by renal metabolism The lower cyclic AMP/creatinine ratio in urine compared with plasma observed in the pres ent work indicates that in the newborn period the renal clearance rates of these two com pounds may be different More work is needed to describe in detail the renal excretion and metabolism of cyclic AMP in the newborn

In LBW infants there was an inverse rela tionship between serum calcium and the uri nary cyclic AMP/creatinine ratio Assuming that urinary cyclic AMP reflects circulating PTH this observation suggests that the para thyroid glands of LBW infants are able to re spond to a lowering of serum calcium even during the first days of life Furthermore it would appear that the tubular receptor mech anism for PTH is mature in newborn LBW infants

The functional state of the parathyroid glands in the early neonatal period is a matter of controversy Measurements of immuno-

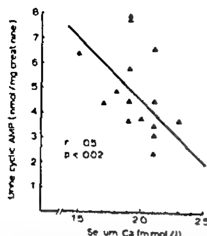


Fig 3 Relation of urine cyclic AMP/creatinine ratio to serum calcium in low birth weight infants For each patient the mean urinary cyclic AMP/creatinine ratio for the first 3 days of life was calculated and related to the lowest serum calcium value observed on these days Each point represents one patient

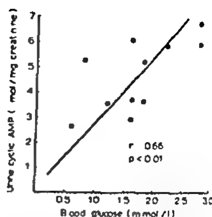


Fig 4 Relation of urine cyclic AMP/creatinine ratio to blood glucose in infants of diabetic mothers Urinary cyclic AMP/creatinine ratio calculated as mentioned in legend to Fig 3 was related to the lowest blood glucose concentration observed on the first 3 days of life Each point represents one patient

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H P	1	41 0	3 3	2 1
	3	81 0	8 3	3 9
K G	1	31 5	4 0	2 8
	3	30 0	4 9	3 6
S N	1	44 5	4 8	3 0
	3	39 2	5 4	5 1

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RENOGRAPHY IN THE FOETAL AND NEWBORN LAMB

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ABSTRACT Hirvonen L. Raty J Kiviniitty K and Timisjarvi J (Departments of Physiology and Radiotherapy University of Oulu Oulu Finland) Renography in the foetal and newborn lamb Acta Paediatr Scand 67 357 1978 —Hippuran renography indicates kidney function as reflected in a substance handled mainly by the tubules The working conditions of the kidneys undergo a fundamental change at birth with the cessation of placental circulation and these organs become responsible for waste elimination [131]I hippuran renography was performed on foetal and newborn lambs using a gamma camera and a computer The intervals of the maximum and half time renal activity were determined These decreased by a half from mean foetal value of $T_m=7$ min and $T_{1/2}=19$ min in 1 to 1 5 days and reached the full-grown level in 2 to 5 weeks No sudden change occurred as a result of the first breath

KEY WORDS Tubular function birth [131]I hippuran gamma camera

Renography with radioactive hippuran indicates kidney function as reflected in a substance handled mainly by the tubules (5) The form of the renogram is influenced by the relative uptake of radioactive hippuran by the kidney the transit time spectrum of the tracer passing through the nephrons the form of the plasma activity curve and the activity present in non renal tissues (9) Certain information is available on human renography during the first weeks and months of life (6 10) and it has been noted that adult standards are soon applicable in the interpretation of the results (7) The working conditions of the kidneys undergo a fundamental change at birth with the cessation of the placental circulation whereafter these organs become responsible for waste elimination Although this may be reflected in renography the magnitude of the change is not known In the experiments reported here renography was performed on lambs before their first breath and during the neonatal period Preliminary reports have been published previously (3 4)

METHODS

Three full term foetuses seven lambs aged 1 hour to 35 days and two full grown Finnish sheep were used Altogether 76 examinations were carried out The animals were given 100 μCi [131]I hippuran intravenously and the renograms then recorded with a gamma camera (Radicamera) with the animal lying on its side In this side projection both kidneys appeared at the same point or close to each other in the gamma camera image (Figs 1 and 2) The results were analyzed by computer (Nukab 2530) Each examination took 30 min and the data were recorded in 20-sec phases the pulses being counted in the area of the kidneys and in that of the bladder with the abdomen serving as the background (Fig 1) The number of pulses per unit surface area was calculated in 0-sec and 1 min intervals When analyzing the renogram the background was subtracted from the activity in the renal area. The time interval from the initial rise of the radioactivity after the injection to the maximum renal activity (T_m) and the halftime for radioactivity ($T_{1/2}$) were determined from the renogram (Fig 3)

A Caesarean section was performed under local anaesthesia and the onset of respiration inhibited by covering the snout with a rubber glove the umbilical circulation being kept intact. In one case the lamb was delivered

reactive PTH have shown low plasma values during the first days of life (7 17), in particular in infants with shortened gestational age (17) Lower than normal PTH values have been observed in hypocalcaemic newborn infants (10) David et al (8) on the other hand concluded from their studies of PTH in premature infants that parathyroid responsiveness was present at birth

In IDMs there was a positive correlation between urinary cyclic AMP and blood glucose This would suggest that glucose production in these infants is dependent upon a process involving cyclic AMP formation Now it is well known that the stimulation by glucagon of hepatic glycogenolysis is mediated by cyclic AMP (5) The effect of glucagon on gluconeogenesis is probably mediated by a similar mechanism (9) On this background it is of interest that a failure of glucagon release has been observed in IDMs (2)

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in human infants aged 1 to 4 days They report a T_m of 4 min (range 1 2 to 9 5 min) and a $T_{1/2}$ of 14 min (7 6 to 25 3 min) In the same way as human babies the lambs seem to reach adult values during the first weeks of life (7 10) Clearance studies indicate that renal function in infants seems to improve and glomerular filtration rate to increase up in the second year of life (2 8) In human subjects renograms of the two kidneys are recorded separately whereas renograms of both kidneys together were recorded here This may partially explain the range of the results Another reason may be the differences in the fluid balance Although the functional conditions of the kidneys change greatly at birth renography indicates that adaptation is gradual This corresponds to haemodynamic changes According to Aperia et al (1) clamping of the cord results in an increase in the perfusion of the outer cortical region of the lamb kidney without any essential change in the total renal blood flow The frequency of the filtering superficial nephrons increases simultaneously

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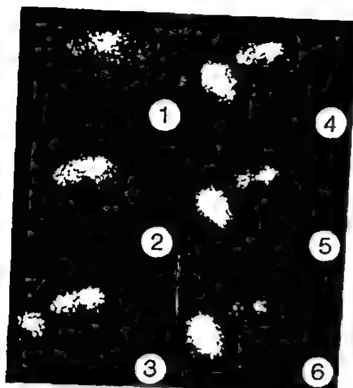


Fig 1 Gamma camera picture of an accumulation of tracer (^{131}I hippuran) in the kidneys and bladder during the 1st (1) 3rd (2) 6th (3) 10th (4) 20th (5) and 30th min (6) The kidneys are located in the upper part of each frame and the bladder on the left side in frames 3 to 6



Fig 2 Television display image indicating the areas marked with a light pen for calculating the renogram and bladder curve 1 kidneys 2 bladder 3 abdominal back ground area

by Caesarean section and allowed to breathe spontaneously It was then examined at the age of 1 hour The other lambs were born spontaneously

RESULTS

The time interval of the maximum renal activity decreased rapidly after birth and reached the full grown value at the age of two to five weeks (Table 1) No sudden change occurred after the first breath however

The mean half time for the radioactivity decreased from 19 min in the foetus to 6 7 min in the 2 to 5 week old lambs and 6 5 min in the full grown animals The decrease occurred in a similar manner to the change in T_m The change from the foetal to the newborn state was a gradual one

Table 1 *T_m and $T_{1/2}$ values grouped by age of animal*

Age	No of animals	T_m (min) Mean	T_m (min) Range	$T_{1/2}$ (min) Mean	$T_{1/2}$ (min) Range
Foetus	3	7	5–10	19	11–26
1 5–5 h	3	6	2–12	13	7–19
1–1 5 d	5	3	2– 4	7 8	5–11
3–6 d	6	3 3	2– 4	8 5	6–14
14–35	7	2	2– 3	6 7	5– 8
Full grown	2	2	2	6 5	5– 8

DISCUSSION

The T_m and $T_{1/2}$ values for the lamb were about equal to those observed by Winter et al (10)

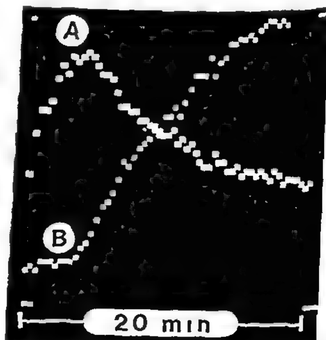


Fig 3 Renogram (*A*) and bladder curve (*B*) of a 10-day old lamb

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THE DIAGNOSIS OF IRON DEFICIENCY BY ERYTHROCYTE PROTOPORPHYRIN AND SERUM FERRITIN ANALYSES

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ABSTRACT Koller M [redacted] Romslo I Finne P H Brockmeier F and Tyssebotn I (Laboratory of Clinical Biochemistry Department of Pediatrics and Department of Physiology University of Bergen Bergen Norway) The diagnosis of iron deficiency by erythrocyte protoporphyrin—and serum ferritin analyses Acta Paediatr Scand 67 361 1978.—Free erythrocyte protoporphyrin (FEP) and serum ferritin have been determined [redacted] 57 healthy children and in 25 children with varying degrees of iron deficiency FEP was found to be inversely correlated to the concentration of hemoglobin ($r = -0\ 80$) as well as to serum ferritin ($r = -0\ 64$) Elevated FEP was found in children with hemoglobin less than [redacted] 5 g/dl or serum ferritin less than 8 μg/l In a group of apparently hematologically normal children between the age of 10–14 years (hemoglobin>12 5 g/dl) a 2-month trial of iron medication resulted in an increase in hemoglobin and ferritin and a decrease in FEP indicating suboptimal supply of iron for hemoglobin synthesis before iron medication In a patient with iron deficiency (FEP 15.3 μmole/l hemoglobin 5.2 g/dl) iron therapy was followed by a rapid fall in FEP before any changes in hemoglobin serum iron transferrin saturation and ferritin could be detected The rapid fall in FEP during start of treatment [redacted] iron deficiency makes FEP a sensitive biochemical parameter on iron homeostasis in iron deficiency anemia

KEY WORDS Free erythrocyte protoporphyrin serum ferritin iron deficiency anemia

Iron deficiency [redacted] probably one of the most widespread nutritional deficiencies in developed as well as in developing countries (29) Although advanced knowledge of infant feeding has eliminated many of the problems of inadequate nutrition iron deficiency and the most significant result hypochromic microcytic anemia [redacted] still a major pediatric problem (28)

Until recently the diagnosis of iron deficiency was based mainly on 1) decreased or absent bone marrow iron 2) serum iron transferrin saturation below 15% and 3) a hypochromic microcytic anemia (17) Depending on how many of these criteria that were present the iron deficiency has been divided into prelatent latent and manifest (17)

In animals [redacted] has been shown that the synthesis of iron proteins other than hemoglobin may become affected early in the development of iron depletion (9) Concurrently abnormalities in cell morphology and cell function occur (9) The reversibility of these abnormalities [redacted] not readily detected by the usual laboratory tests The possibility therefore exists that iron deficiency may persist although the commonly accepted indices of iron deficiency anemia i e hemoglobin and serum iron transferrin saturation have returned to normal levels Thus in the growing child who normally delicately balances its iron requirement and supply (28 29) it is of great importance to diagnose iron deficiency at the prelatent (or latent) stage that [redacted] before the typical hypochromic anemia appears and before the deficiency has resulted in any subcellular damage (7 9) So far however the diagnosis of prelatent iron deficiency mainly rests on decreased or absent stainable iron in bone marrow biopsies (17) and for technical reasons therefore a more simple and

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THE DIAGNOSIS OF IRON DEFICIENCY BY ERYTHROCYTE PROTOPORPHYRIN AND SERUM FERRITIN ANALYSES

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Table 1 *Hematological and biochemical data in 57 healthy children of both sexes aged 10–14 years*

	Mean±S D
Hemoglobin (g/dl)	13 7±0 7
Erythrocyte protoporphyrin (µmole/l)	1 14±0 23
Ferritin (µg/l)	17 ±7
Serum iron (µmole/l)	18 5±6 5
Serum iron binding capacity (µmole/l)	73 2±8 2
Blood lead (µmole/l)	0 89±0 21

equally reliable method to detect iron depletion would be welcome

In recent years there has been renewed interest in the analysis of free erythrocyte protoporphyrin (FEP) as an additional tool to diagnose suboptimal iron availability for heme synthesis (6 8 18 19 21–24 27) The rationale behind the FEP analysis is that in absolute or relative iron depletion protoporphyrin accumulates within the erythroid cells as long as the porphyrin biosynthesis remains intact (3 14, 19) It should be noted however that an increase in FEP is not specific to iron depletion Thus in diseases with reduced ferrochelatase activity or in conditions where the synthesis of porphyrins outstrips an otherwise normal iron supply there is an increase in FEP as well (4, 11) Compared to iron deficiency these are relatively rare diseases and they would therefore scarcely invalidate the usefulness of the FEP analysis for most practical purposes

Serum ferritin determination is another analysis that has been advocated as a useful test to diagnose iron depletion (1 26) The usefulness of this analysis depends on the finding that the concentration of serum ferritin is linearly correlated to the amount of depot iron (20)

In the following we report data on FEP and ferritin analyses in healthy children and in children with varying degrees of iron deficiency

MATERIALS AND METHODS

82 children were studied They were divided into two groups

Group I 57 hematologically normal children aged 10–14 years All the children had hemoglobin levels above 12 5 g/dl serum iron transferrin saturation above 15% and erythrocyte protoporphyrin (FEP) less than 1 6 µmole/l (Table 1)

Group II 25 children aged 7–14 years with iron deficiency Eighteen children were anemic (hemoglobin<12 0 g/dl) and 7 had hemoglobin levels between 12–13 g/dl All had FEP>1 8 µmole/l and 18 had ferritin <10 µg/l None of the children (except patients A L and L E see below) had clinical or laboratory evidence of diseases other than iron deficiency

The results from two patients are presented in more details (see Figs 2 and 3)

Blood was collected by venipuncture after an overnight fast and when necessary anticoagulated with EDTA Blood counts and red cell indices were performed on a Coulter Model S Red cell morphology was examined on May Grunewald-Giemsa stained blood smears

Serum iron and serum iron binding capacity were measured as described by Richterich (25)

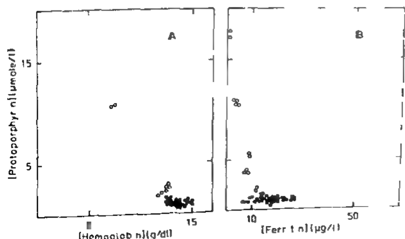


Fig 1 The relationship between the concentration of hemoglobin and free erythrocyte protoporphyrin (A) and between the concentration of free erythrocyte protoporphyrin and serum ferritin (B) in hematologically normal children (●) and in children with iron deficiency (O)

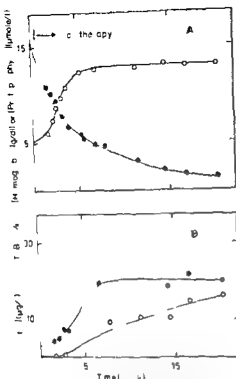


Fig 2 Time course of the changes in (A) hemoglobin (O) and free erythrocyte protoporphyrin (●) and (B) ferritin (O) and serum iron transferrin saturation (●) in a patient with iron deficiency during treatment (patient A L see text for further details)

Serum ferritin was determined by a commercially available RIA kit (from Ramco Lab Inc Houston Texas U S A)

Lead was determined by flameless atomic absorption spectrophotometry (13) using an Il 453 atomic absorption spectrophotometer equipped with an Il 455 programmer

Erythrocyte protoporphyrin was determined fluorometrically as described by Piomelli et al (3)

The day to-day coefficients of variation of the methods were (%) hemoglobin ? I FEP 4 9 serum iron ? 0 serum iron binding capacity 3 0 ferritin ? 6 and lead ? ?

RESULTS

Table 1 shows the values obtained for FEP hemoglobin lead ferritin serum iron and serum iron binding capacity in the children of group I There were no significant differences between the values obtained in girls and boys (data not shown) and except for ferritin the results were all within generally accepted limits (5 26 27)

Fig 1A demonstrates the relationship between FEP and hemoglobin concentration in groups I and II For group II the hemoglobin concentration was found to be inversely related to the concentration of FEP ($r=-0$ 80) and of 7 children with hemoglobin between 12–13 g/dl 6 had FEP above 2 0 μmole/l In group I (hemoglobin>12 5 g/dl) all the children had FEP less than 1 8 μmole/l

None of the children in either group had blood lead levels above 1 4 μmole/l (data not shown)

Fig 1B demonstrates the correlation between ferritin and FEP An inverse relationship between FEP and ferritin among children was found in group II ($r=-0$ 64) while in group I this could not be demonstrated

Fig 2 summarizes the findings in patient A L an 11 year old girl with iron deficiency anemia secondarily to Peutz Jeghers syndrome On peroral iron therapy there was a rapid decline in FEP a slightly delayed increase in iron transferrin saturation and hemoglobin and an even more delayed rise in serum ferritin Hemoglobin and serum iron transferrin saturation reached normal limits long before FEP was normalized

Fig 3 summarizes the findings in patient L E who was a 4 year old girl admitted to the out patient clinic because of anemia and failure to thrive The diagnosis iron deficiency was made and peroral iron therapy started However due to the lack of effect of iron therapy on the hemoglobin level and the minimal effect on FEP the patient was further examined and a coeliac disease was diagnosed On a gluten free diet supplemented with iron in the same amount as previously an increase in hemoglobin and a decrease in FEP appeared Note here that FEP started to decline when the patient was given iron alone and before there were any significant changes in the concentration of hemoglobin Note also that when the patient was given a gluten free diet the very marked decrease in FEP preceeded the increase in the concentration of hemoglobin

The concentration of serum ferritin in our

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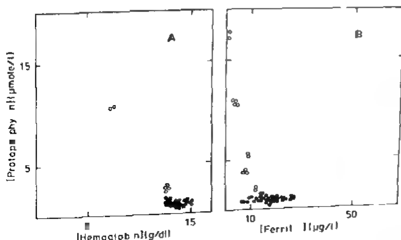


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and/or absorbed (Fig 3) Thus the sensitivity of FEP to detect disturbances in the supply of iron apparently surpasses that of hemoglobin and serum iron transferrin saturation This conclusion is in agreement with the findings of McLaren et al (21) and Piomelli et al (23)

According to Piomelli et al (23) and Langer et al (19) in iron deficiency FEP never exceeds 500 μg/dl (equivalent to 8 9 μmole/l) However as shown in Figs 2 and 3 FEP may surpass 15 μmole/l in advanced iron deficiency with normal serum lead concentration and without the photosensitivity characteristic of erythropoietic protoporphyria Essentially similar results were reported in a recent study by Thomas et al (27) Thus it is not justified to state an upper limit of FEP of approx 9 μmole/l to discriminate between iron deficiency lead intoxication and erythropoietic protoporphyria (23)

With respect to ferritin this parameter is less suited than FEP to detect rapid changes in the balance between the demand and the supply of iron to the erythron (27) This depends partly on the fact that ferritin is an acute phase reactant (27) and partly on the finding that serum ferritin reflects the iron store not the dynamic balance between the iron supply and demand in the erythron (27) In agreement with these observations we found ferritin values less than 10 μg/l mostly paired with greatly elevated FEP values

ACKNOWLEDGEMENTS

We are grateful to Dr P Skagseth and Dr G Fluge for allowing us to study children under their care

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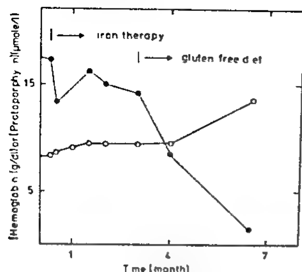


Fig 3 Time course of the changes in hemoglobin (O) and free erythrocyte protoporphyrin (●) in a patient with iron deficiency secondarily to coeliac disease (patient L E see text for further details) The patient was given iron (30 mg ferrous iron/day) supplemented with a gluten free diet as indicated

group I (Table 1) is significantly below that reported in studies from other laboratories (1 5 23) Part of this discrepancy could be ascribed to methodological differences e g the specificity of the antihuman antiferritin (12) The possibility exists however that the apparently healthy children with normal hemoglobin levels had suboptimal iron stores Thirty one of the children in group I were selected at random and given a daily dose of 200 mg iron (as ferrous sulphate) for 8 weeks As seen from Fig 4 of the paired determinations there was an increase in hemoglobin in 26 a decrease in FEP in 30 and an increase in ferritin in 30 children respectively The average per cent changes were hemoglobin +4 5 FEP −18 and ferritin +130 The changes were statistically significant ($p<0.01$)

DISCUSSION

The present study demonstrates an inverse relationship between hemoglobin and FEP in iron deficiency (Fig 1 A) Essentially similar results have been reported by Dagg et al (8) Langer et al (19) and Thomas et al (27)

An increase in FEP is found also in the anemias of chronic infections (3 19) and leukemia (3) In these disorders the increase in FEP may be ascribed to a defective reutilization of iron (15 16) In contrast in the anemias of acute infections or in pernicious anemia there is no change in FEP (3) Thus except for lead intoxication (4) and erythropoietic protoporphyria (11) elevated FEP is generally associated with hypoferremia and a normoblastic bone marrow This conclusion is in keeping with the data of Fig 1B i e at ferritin concentrations less than 10 µg/l (which has been shown to coincide with decreased or absent bone marrow iron (21 22)) there is a marked increase in FEP

Of considerable interest is the finding that in children of group I (selected according to ref 2) iron medication results in a decrease in FEP and an increase in hemoglobin and ferritin (Fig 4) As shown by Dagg et al (8) and McLaren et al (21) these findings are typical of sideropenia i e the children of our reference material may have suboptimal iron stores This suggestion is supported also by the finding of a mean serum ferritin level (Table 1) significantly below that reported in healthy children by other workers (26)

In iron deficiency anemia iron therapy is followed by a decrease in FEP within 2–3 days if appropriate amounts of iron are given (Fig 2)

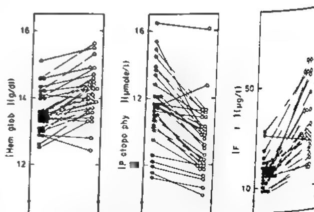


Fig 4 Hemoglobin free erythrocyte protoporphyrin and ferritin levels in hematologically normal children before (●) and after (O) 8 weeks on peroral iron therapy (200 mg ferrous iron/day)

EFFECTS OF THIAMINE IN A PATIENT WITH A VARIANT FORM OF BRANCHED CHAIN KETOACIDURIA

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ABSTRACT Duran M Tielens A G M Wadman S K (University Children's Hospital Het Wilhelmina Kinderziekenhuis Utrecht) Stigter J C M (St Franciscus Gasthuis Rotterdam) and Kleijer W J (Department of Cell Biology and Genetics Erasmus University Rotterdam The Netherlands) Effects of thiamine in a patient with a variant form of branched-chain ketoaciduria Acta Paediatr Scand 67 367 1978.—A boy with the intermediate variant of branched-chain ketoaciduria was studied Treatment with an amino acid mixture was discontinued at the age of 7 5 years Reintroduction of normal protein containing foods precipitated the biochemical abnormalities characteristic of MSUD which were relieved by 10 mg thiamine/day Adaptation to this regimen developed and thiamine intake was increased to 100 mg/day later to 1 000 mg/day The patient developed well and had no attacks of ketoacidosis 1-^{14}C leucine degradation in intact fibroblasts was 15% of controls and did not increase upon addition of thiamine to the incubation medium

KEY WORDS Branched-chain ketoaciduria MSUD variant thiamine therapy

Several different forms of branched chain ketoaciduria or maple syrup urine disease (MSUD) have been distinguished These include the classical form with rapid fatal outcome the intermittent form with bouts of ketoaciduria (possibly caused by infections) (2) the intermediate form in which practically no periods of acute illness occur (13) and a thiamine responsive variant in which the biochemical abnormalities are reversed by oral supplements of pharmacological doses of thiamine (14) Both the classical and variant forms are characterized biochemically by a *deficiency* in branched-chain ketoacid (BCKA) decarboxylase *activity In the* variant forms somewhat higher BCKA decarboxylase activities have been *found in* cultured fibroblasts as compared with *the classical form*

In this report we describe the clinical progress and some biochemical results in a patient whose case history has previously been described (8)

Initially at the age of 18 months this patient showed many signs of MSUD although less severe than in the classical form vomiting acidosis mental retardation and neurological manifestations Subsequently he was treated with a mixture of amino acids containing 33 mg leucine 18 mg isoleucine 27 *mg valine* per kg bodyweight and 1 5 mg thiamine On this regimen a normal physical and intellectual development was observed during *the next six* years Because of his good progress we tried to replace the amino acid mixture by a diet consisting of normal protein containing food combined with pharmacological doses of thiamine as used by Scriver et al's (14) Criteria for adequate treatment should be no recurrence of excessive amounts of toxic metabolites not even in states of disease and

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Abbreviations MSUD=*maple syrup urine disease* BCKA=branched-chain ketoacids BCAA=branched chain amino acids

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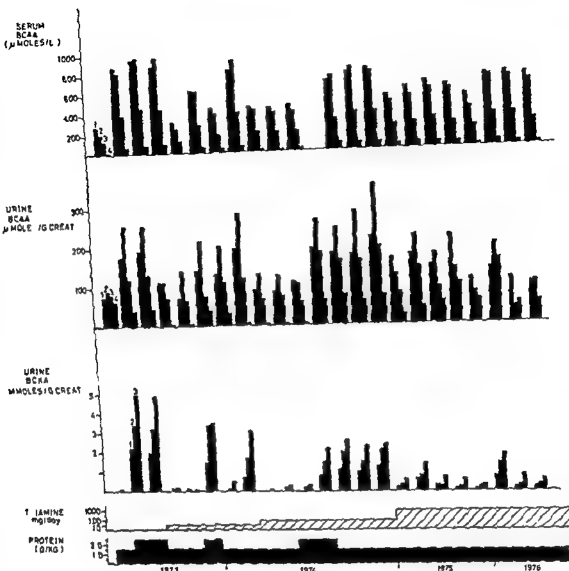


Fig. 2. Time-course of urinary *branched-chain ketoacids* (BCKA. 1=α ketoisovaleric acid. 2=α ketoisocaproic acid. 3=α keto-β methylvaleric acid), branched-chain *amino acids* (BCAA. 1=valine. 2=leucine. 3=isoleucine. 4=*allo*-isoleucine) and serum BCAA with respect to thiamine therapy and protein intake.

Normal upper limits: urine: BCKA not detected; valine 60 μmol/g creat.; leucine 9? μmol/g creat.; isoleucine 76 μmol/g creat.; *allo*-isoleucine not detected. Serum: valine 310 μmol/l; leucine 214 μmol/l; isoleucine 89 μmol/l; *allo*-isoleucine not detected.

Initially, an almost complete biochemical normalization took place as is shown by the 5th analysis made 21 days after thiamine therapy was started. However, biochemical abnormalities reappeared in the next few months. These could not be reversed by a further restriction of the protein intake. After an attack of aminoacidemia and ketoaciduria (8th analysis) the daily thiamine supplement was raised to 100 mg. No clinical or biochemical derangements occurred in the following months, despite a protein intake of 2.5 g/kg

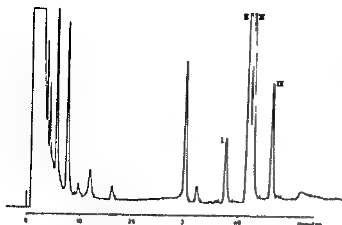


Fig 1 Gas chromatogram of urinary α ketoacids (TMS quinoxalinol derivatives) in patient W B I α ketoisovaleric acid II ■ keto-β methylvaleric acid III α ketoisocaproic acid IV ■ ketocaproic acid (internal standard) Injected was a volume corresponding with 0 01 ml of urine

absence of neurological manifestations of any kind

The effect of treatment was followed by regular determinations of urinary BCKA and BCAA as well as amino acids in fasting serum For a further characterization we quantified the enzyme defect in the patient s cultured fibroblasts

METHODS

Amino acids Quantitative amino acid determinations in fasting serum and 24 h urine samples were performed with the standard Technicon TSM I method for physiological fluids

Gas chromatography of branched chain ketoacids Alpha ketoacids were analyzed as quinoxalinol derivatives (11) by a modified procedure To 5 ml of urine (containing 0 5 mg of internal standard α ketocaproic acid sodium salt) was added 5 ml of a freshly prepared solution of 100 mg/ml of o-diaminobenzene in 2 N HCl The mixture was incubated overnight in the dark at room temperature Subsequently the solution was extracted three times with 25 ml of chloroform The combined extracts were dried over anhydrous sodium sulphate and after decantation evaporated to dryness under reduced pressure (40°C) Trimethylsilylation of the compounds was done with N O bis(trimethylsilyl)acetamide in dry pyridine (7 1 v/v) at room temperature during 1 hour The efficiency of the quinoxalinol formation ranged from 90–100% The TMS derivatives were analyzed on a Becker 420 gas chromatograph equipped with dual (2 4 m×3 mm) stainless steel columns which were packed with 5% SE 52 on Chromosorb W AW DMCS 100–120 mesh Temperature 100°C isothermal for 10 min then programming to 200°C at a rate of 2 5°C/min Variation coefficients were less than 6% for all acids The identi of the formed products was confirmed with gas chromatography–mass spectrometry (GC-MS) A typical g chromatogram of a urine extract from our patient is show in Fig 1

Cell culture Fibroblasts were cultured by standa methods from skin biopsies of patient W B three pa tients having classical MSUD and a control individua The growth medium was Ham s F 10 supplemented wi 15% fetal calf serum (Flow company) penicillin (50 U/m and streptomycin (50 μg/ml) The fibroblast strains we used for assays in their 6th–12th passages

Branched-chain α-ketoacid decarboxylase assay Fi broblasts were harvested by trypsinization (0 25% try sin in physiological saline) washed twice in Kreb Ringer phosphate buffer pH 7 2 with 2 mmol/l glucos and resuspended in the same buffer solution to a ce density of 400000 cells/ml From the cell suspensio samples were taken simultaneously for a protein assa according to Lowry et al (12) and for the decarboxylas assay Samples of 50 μl (i e 20000 cells correspondi with 7 μg of protein) were pipetted in the wells (diamet 7 mm) of tissue culture plates (Micro Test II Falco and followed by the addition of 50 μl of a freshly ma solution containing 0 01 μmol (0 6 μCi) 1 ^{14}C leuci (specific activity 60 mCi/mmol Radiochemical Centr Amersham U K) in Krebs Ringer phosphate buffer p 7 2 with 20 mmol/l glucose and 50 mmol/l thiamine HC Reaction blanks consisted of an equal number of cel which had been placed in a boiling waterbath for 5 mi The wells were covered with glass fibre filters (diamet 10 mm AP 25000 Milipore Brussels) soaked in 3 5 NaOH as described by Wendel et al (16) and the plat were incubated for 2 hours at 37°C After incubation t filters were transferred to scintillation counting vials in 10 ml of liquid scintillation cocktail (Dimulume 3 Packard) were added Radioactivity was counted in Packard Tricarb scintillation spectrometer at an efficienc of 90% Boiled cell blanks yielded about 140 cpm/2 h

RESULTS

After discontinuing the treatment with a amino acid mixture the patient was placed o a diet consisting of normal foods in which th protein content was 1 5 g/kg correspondin with 126 mg leucine 77 mg isoleucine and 8 mg valine per kg bodyweight Attempts wer made to raise the protein intake to 2 5 g/kg At first the patient showed a slight aversio against protein containing foods but this aver sion soon disappeared On introduction o normal foods a striking increase of both urinary BCKA and BCAA was seen (Fig 2) Then the thiamine trial was started with daily amounts of 10 mg

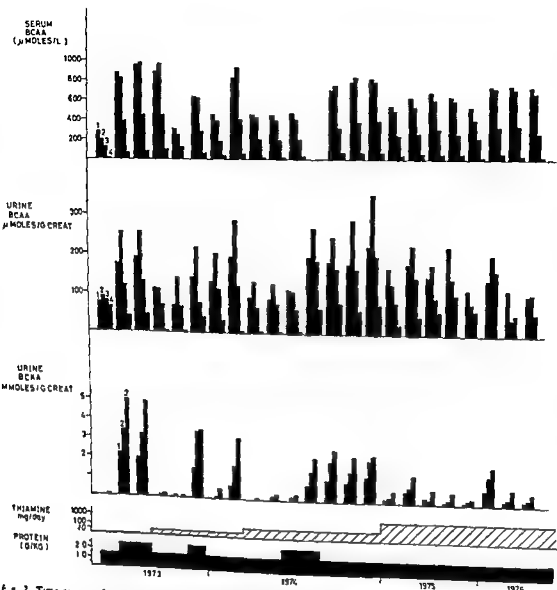


Fig. 2. Time-course of urinary branched-chain ketoacids (BCKA: 1=α ketoisovaleric acid, 2=α ketoisocaproic acid, 3=α keto-β methylvaleric acid), branched-chain amino acids (BCAA: 1=valine, 2=leucine, 3=isoleucine, 4=*allo*-isoleucine) and serum BCAA with respect to thiamine therapy and protein intake.

Normal upper limits: urine BCKA not detected; valine 60 μmol/g creat; leucine 92 μmol/g creat; isoleucine 76 μmol/g creat; *allo*-isoleucine not detected. Serum: valine 310 μmol/l; leucine 214 μmol/l; isoleucine 89 μmol/l; *allo*-isoleucine not detected.

Initially an almost complete biochemical normalization took place as is shown by the 5th analysis made 21 days after thiamine therapy was started. However, biochemical abnormalities reappeared in the next few months. These could not be reversed by a further restriction of the protein intake. After an attack of aminoacidemia and ketoaciduria (8th analysis) the daily thiamine supplement was raised to 100 mg. No clinical or biochemical derangements occurred in the following months, despite a protein intake of 2.5 g/kg

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The combination of protein restriction and large doses of thiamine has now been given for over two years BCKA excretions never reached the pre thiamine levels On the other hand the almost completely normal metabolite pattern as was observed on the amino acid mixture diet did not return Plasma BCAA concentrations were always elevated to some extent and *allo* isoleucine was always present

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During the period of observation we frequently observed hyperlactatemia (up to 7 7 mmol/l normal <2 0 mmol/l) Also serum uric acid was elevated (0 47 mmol/l normal 0 12–0 35 mmol/l) Metabolic acidosis as measured with the Astrup method was never observed This discrepancy between organic acidemia and base excess has been observed before (1)

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The treatment of branched chain ketoaciduria consists of a drastic reduction of the intake of leucine isoleucine and valine Dancis et al (3) made a classification of MSUD types based on the difference in residual decarboxylase activities in cultured skin fibroblasts It was suggested by these authors that dietary treatment is not necessary in the intermediate forms with activities >8%

In contrast dietary treatment was clearly needed for our patient whose fibroblasts degraded ^{14}C leucine at a rate of 15% of controls A remarkable clinical improvement was observed after the start of the diet disappearance of the ataxia no recurrence of ketoacidotic attacks and catch up of psychomotor development It seems questionable whether enzyme activity in cultured fibroblasts sufficiently reflects the metabolic capacity of the liver only The latter determines the need for treatment

Few reports have been published on the effects of discontinuing the dietary treatment in MSUD We tried to stop the diet in our patient because of his excellent progress

Introduction of normal foods was rapidly followed by oral supplements of thiamine An almost instantaneous decrease of BCKA excretion was achieved on 10 mg thiamine/day However a gradually decreasing effect of thiamine indicated some adaptation which might be due to an induced endogenous break down of thiamine

The effect of thiamine in our patient to reduce BCKA and BCAA excretions and serum BCAA levels has also been observed in other patients (5 9 14) However the adaptation to thiamine which necessitated an increase of the thiamine to 1 000 mg/day has not been described by the other authors Elsas et al (5) and Kodama et al (9) gave rather high amounts of thiamine (100–150 mg/day) but their studies did not last for more than one or several weeks The findings in our patient are in agreement with Elsas et al (5) and Kodama et al (9) who demonstrated that thiamine resulted in a more pronounced decrease of BCKA-excretion as compared with the effect on serum BCAA levels Kodama s patient showed a marked clinical improvement accompanied by disappearance of BCKA from the urine already after thiamine therapy but plasma BCAA normalized only after starting a diet consisting of an amino acid mixture In our patient comparable results were obtained although the sequence of therapeutic measures was reversed

Studies on the effects of thiamine in *in vitro* cultured fibroblasts of the patient did not show any stimulation of the decarboxylation of 1 ^{14}C leucine The interpretation of this result is however hampered by the lack of data on the ability of thiamine to enter the cells and to increase the intracellular thiamine level formed during previous growth in culture medium which contained 1 mg thiamine/l

The *in vivo* effect of thiamine might be the result of an activation of hepatic BCKA-decarboxylase as was described by Danner et al in normal subjects after at least 18 days on 100 mg/day thiamine (4) This might offer an attractive and more generally applicable therapeutic possibility for the treatment of MSUD especially in older children who are candidates for a protein restricted diet

The ketoacid pattern in our patient was of the type found in patients with variant forms of the disease (7 10) the ketoacid derived from isoleucine was higher than that of leucine In classical MSUD α ketoisocaproic acid is the most prominent metabolite (10) It seems attractive to postulate that a good response to thiamine may only be expected in those patients who have α keto-β methyl valeric acid as the most prominent ketoacid More evidence in this direction was obtained by Singh et al (15) who showed that only the degradation of isoleucine could be stimulated by thiamine in disrupted fibroblasts

In our opinion the determination of urinary BCKA is the most sensitive parameter in the management of MSUD-patients Changes in therapy are best guided by the excretion of these acids

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Acta Pædiatr Scand 67 373–377 1978

VISUAL DISORDERS IN 7 YEAR OLD CHILDREN WITH AND WITHOUT PREVIOUS VISION SCREENING

LENNART KÖHLER and GÖRAN STIGMAR

From the Departments of Paediatrics and Ophthalmology University Hospital Lund Sweden

ABSTRACT Kohler L and Stigmar G (Departments of Paediatrics and Ophthalmology University Hospital Lund Sweden) Visual disorders [redacted] 7 year-old children with and without previous vision screening Acta Paediatr Scand 67 373 1978.—An analysis [redacted] visual defects among 310 children referred from a vision screening of 2 178 7 year-old children revealed a 50 % frequency of significant eye defects among the referrals (7 % of screened children) Of the screened children one group (1 530 children) had previous visual screening three years earlier The other group (648 children) had no previous vision screening until the age of seven A comparison between the two groups showed that the risk of finding a new significant eye disorder in a school entrant was more than 6 times greater for a child who was not examined in his preschool years and the risk of finding an amblyopic child was more than 10 times greater The results do indicate the need for continuation of the present vision screening program of pre-school children

KEY WORDS Vision screening school children pre school children amblyopia strabismus

Screening for visual defects is routine in many preeschool and school health programmes Reported results however show great disparity obviously due to biased samples differences in screening methods in criteria for referral and in professional evaluation Proper evaluation of the screening methods are rare e g by using control groups or by reexamining at a later age previously screened children

This study of an unselected population of 7 year-old school entrants in a community in Southern Sweden was performed in order to

(1) identify children with impaired visual function previously undetected or insufficiently treated

(2) describe these disorders in terms of need for professional care

(3) evaluate the eye screening carried out 3 years earlier in the same community (5)

By comparing the number and significance of eye disorders in children previously eye screened with those in children not previously eye screened the importance of pre school vision screening could be elucidated

MATERIAL

During 3 years 2 178 7 year-old children born 1963–1965 started school [redacted] Lund Of these 1 530 (70·2%) were previously vision screened at the age of 4 years 558 (25·5%) had moved into the city between 4 and 7 years of age 60 (2·8%) had failed to attend the health control and 30 (1·4%) were incompletely vision screened at 4 years Complete data regarding the visual acuity in the screening procedure [redacted] school were available only for children born 1963 and 1965

METHODS

The monocular visual acuity of all children was tested by the school nurses in their respective schools using a linear E-chart (Oculus) at a distance of 5 m Children with a visual acuity of 0·9 or less in one or both eyes i e

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[1] 1·0 is equivalent to 6/6 0·6 to 6/10 0·2 to 6/30 0·1 to 6/60

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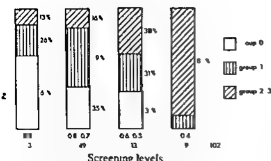


Fig 1 Correspondence between ophthalmological classification and different screening levels of visual acuity in 10 7 year-old children

The classification of the 310 children in terms of need of professional care is presented in Table 2

Altogether 49% of the referred children (7% of all screened) had significant eye disorders and needed treatment Correction with glasses was prescribed in 133 children (6 1%) Overreferral (group 0) was found in 21 9% (3 1% of all screened)

The criterion for referral from the visual screening was a visual acuity of 0 9 or less Among the newly detected children born 1963 and 1965 there was a direct correlation between low visual screening acuity and significant eye disorders (r=0 51) (Table 3 Fig 1) This correlation is highly significant

It was also found that if lower criteria for referral had been used the rate of overreferral would have diminished but at the same time fewer children with need of professional care would have been detected (Table 3 Fig 1)

Among the 310 children referred from the screening 40 had newly detected significant eye disorders (Table 4) Eleven of these were previously eye screened at 4 years of age with normal result and thus had either developed their eye disorder between 4 and 7 years of age or were missed when screened at 4 years

The most frequent diagnosis among these eleven children was simple myopia (>−1 0 D) occurring in 5 children and hyperopia (>+1 5 D) with visual impairment in 3 children Intermittent strabismus without amblyopia was noted in 2 children and functional amblyopia secondary to anisometropia in one child

The remaining 29 children out of the 40 with newly detected significant eye disorders were not previously vision screened The most common causes of their visual impairment were hyperopia and astigmatism (17 out of 29) Simple myopia occurred in 5 children strabismus without amblyopia in 2 children and functional amblyopia in 5 children

DISCUSSION

The primary and most important objective of vision screening should be to determine the presence of impaired vision by a rapid yet accurate screening test and thereafter to elicit the cause of the defect at a more detailed ophthalmologic examination In this survey the only screening method used has been an assessment of distant visual acuity By this method some children with hyperopia strabismus and other disturbances of binocular vision may pass the examination as false-negative and yet have defects in need of treatment

However it was felt that it would be impractible to conduct a more extensive screening program in view of the large number of children involved Besides in a previous study of 4 year old children the majority (97%) of eye disorders were detected by the visual acuity test only (5) Other screening methods

Table 3 *Ophthalmological classification and screening levels of visual acuity in 102 7 year old children with newly detected eye disorders*

Screening level of poorest eye	Ophthalmological classification				Total
	0	1	2	3	
0 9	19	8	4	0	31
0 8–0 7	17	?4	7	1	49
0 6–0 5	4	4	5	0	13
≤0 4	0	1	5	3	9
Total	40	37	21	4	10?
	77		?5		

Table 1 *Ophthalmological examination of 310 7 year old children referred from eye screening*

	n	% of referred (n=310)	% of screened (n=2 178)
Visual acuity of poorest eye			
1 0–0 7	237	76 5	10 9
0 6–0 2	66	21 3	3 0
≤0 1	7	2 2	0 3
Total	310	100 0	14 2
Functional amblyopia	18	5 8	0 8
Refraction			
Astigmatism >1 5 D	83	26 8	3 8
Hyperopia >1 5 D	87	28 1	4 0
Myopia >1 0 D	40	12 9	1 8
No or slight refractive errors	100	32 2	4 6
Total	310	100 0	14 2
Anisometropia >1 0 D	16	5 2	0 7
Position of the eyes			
Esotropia	38	12 2	1 7
Exotropia	3	1 0	0 1
No tropia	269	86 8	12 4
Total	310	100 0	14 2

failing more than 2 symbols on the actual line were referred to the ophthalmologist for further evaluation

Children under current ophthalmological care were referred only if they failed the criteria

The diagnostic investigation of the referred children included the following items performed by two senior ophthalmologists according to a standardized scheme

1 Visual acuity test The monocular visual acuity was checked on a projector chart at 5 m (Rodavist® il literate E letters in a row) The row with the smallest optotypes which the child could read was recorded
2 Cover test for near and for distant vision
3 Inspection of ocular movements
4 Worth s 4 dot test the classical Worth s test was used for distant and a modified test with pictures for near vision
5 Stereo acuity tests examined only for near vision with the Wirth Polaroid tests
6 Determination of the refractive state by retinoscopy 45 min after installation of cycloplegic drops (Cyclogyl® 1%)
7 Examination of the ocular media and fundus

In most cases the 4 dioptre prism test was carried out In the presence of amblyopia the fixation was revealed by the visuscope

The results of the professional examination were expressed in a conventional way based on visual acuity refraction and position of the eyes However we attempted to evaluate the ophthalmological findings also in terms of need for professional care The following classification was used

Group 0 Normal eye examination

Group 1 Mild visual defects without need of treatment Examples Myopia ≤−1 0 D hyperopia ≤+1 5 D astigmatism ≤±1 5 D slight heterophorias without symptoms

Group 2 Refractive visual disturbances exceeding limits for group 1 but without functional amblyopia Treatment indicated Heterophorias with symptoms

Group 3 Functional amblyopia of various origin Strabismus

Groups 2 and 3 were called *significant eye disorders* and were considered as functionally important health problems in the same way as in the health control of 4 year-olds (5)

RESULTS

Out of the 2 178 children screened in school 310 (14 2%) were referred for further evaluation These children were classified according to their state of visual acuity, refraction and eye muscle balance respectively (Table 1)

Visual acuity of ≤0 6 was found in 23 5% (3 3% of all tested) and ≤0 1 in 2 2% (0 3% of all) Functional amblyopia secondary to strabismus or anisometropia (2) was diagnosed in 5 8% (0 8% of all)

Manifest strabismus was found in 13 2% (1 8% of all) The main errors of refraction were hyperopia (28 1% 4 0% of all) and astigmatism (26 8% 3 8% of all) while myopia in this age still was not that frequent (12 9% 1 8% of all)

Table 2 *Classification of 310 7 year old children in terms of need for ophthalmological care*

	n	% of referrals (n=310)	% of screened (n=2 178)
Ophthalmological classification			
0	68	21 9	3 1
1	90	29 0	4 1
2	113	36 5	5 2
3	39	12 6	1 8
Total	310	100 0	14 2
Significant eye disorders (2+3)	152	49 0	7 0

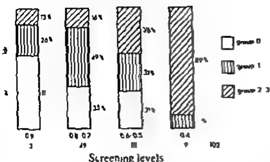


Fig 1 Correspondence between ophthalmological classification and different screening levels of visual acuity in 10? 7 year-old children

The classification of the 310 children in terms of need of professional care is presented in Table 2

Altogether 49% of the referred children (7% of all screened) had significant eye disorders and needed treatment Correction with glasses was prescribed in 133 children (6 1%) Overreferral (group 0) was found in 21 9% (3 1% of all screened)

The criterion for referral from the visual screening was a visual acuity of 0 9 or less Among the newly detected children born 1963 and 1965 there was a direct correlation between low visual screening acuity and significant eye disorders (r=0 51) (Table 3 Fig 1) This correlation is highly significant

It was also found that if lower criteria for referral had been used the rate of overreferral would have diminished but at the same time fewer children with need of professional care would have been detected (Table 3 Fig 1)

Among the 310 children referred from the screening 40 had newly detected significant eye disorders (Table 4) Eleven of these were previously eye screened at 4 years of age with normal result and thus had either developed their eye disorder between 4 and 7 years of age or were missed when screened at 4 years

The most frequent diagnosis among these eleven children was simple myopia (>−1 0 D) occurring in 5 children and hyperopia (>+1 5 D) with visual impairment in 3 children Intermittent strabismus without amblyopia was noted in 2 children and functional amblyopia secondary to anisometropia in one child

The remaining 29 children out of the 40 with newly detected significant eye disorders were not previously vision screened The most common causes of their visual impairment were hyperopia and astigmatism (17 out of 29) Simple myopia occurred in 5 children strabismus without amblyopia in 2 children and functional amblyopia in 5 children

DISCUSSION

The primary and most important objective of vision screening should be to determine the presence of impaired vision by a rapid yet accurate screening test and thereafter to elicit the cause of the defect at a more detailed ophthalmologic examination In this survey the only screening method used has been an assessment of distant visual acuity By this method some children with hyperopia strabismus and other disturbances of binocular vision may pass the examination as false–negative and yet have defects in need of treatment

However it was felt that it would be impractible to conduct a more extensive screening program in view of the large number of children involved Besides in a previous study of 4 year-old children the majority (97%) of eye disorders were detected by the visual acuity test only (5) Other screening methods

Table 3 *Ophthalmological classification and screening levels of visual acuity in 102 7 year old children with newly detected eye disorders*

Screening level of poorest eye	Ophthalmological classification				Total
	0	1	2	3	
0 9	19	8	4	0	31
0 8−0 7	17	24	7	1	49
0 6−0 5	4	4	5	[illegible]	13
<0 4	0	1	5	3	9
Total	40	37	21	4	102
	77		25		

Table 4 *Newly detected significant eye disorders in 7 year old children with and without eye screening at 4 years of age*

	Newly detected significant eye disorder		No newly detected significant eye disorder		Sum	
	n	%	*n*	%	*n*	%
Previously eye screened at 4 years of age	11	0 7	1 519	99 3	1 530	100
Previously not eye screened	29	4 5	619	95 5	648	100
Total	40	1 8	2 138	98 2	2 178	100

(cover test stereo test) revealed very few additional children with significant eye disorders and therefore these tests were omitted in the present survey Also children with eye symptoms like blurred vision eye strains are likely to present themselves to the school health services from where they are normally referred for a professional eye examination

For the assessment of visual acuity, an illiterate E letter chart was used It is recommended for use in Swedish schools as an accurate and easily learned method for testing visual acuity at this age (10) The screening level for referral (a visual acuity of 0 9 or less) was set rather high, since it was our ambition to find and assess also slight visual impairments which might be of importance for these school entrants

Since children under adequate ophthalmological care were not referred the figures in Table 1 do not reflect the prevalence of eye disorders among school entrants, but they present children with not fully treated or corrected visual impairments

In the professional evaluation of the referred children consideration was taken not only of the visual acuity but also of the objective refraction and of the state of binocular vision and symptoms Based on these principles a classification into 4 groups was performed in an attempt to express the findings in terms of need for professional care in the same way as was done previously at the age of 4 years (5) However, since the children were 3 years older and the demands of visual ability were increased in school some modifications of the grouping criteria were introduced Thus the lower limit of significant hyperopia was set as +1 5 C compared with +4 D at 4 years of age

Although the treatment of strabismus and amblyopia is more favourable at an earlier age (7) there are still possibilities of successful results if treatment of children with these defects is started around 7–8 years of age (2 6) Also at this age strabismus and amblyopia should therefore be considered as important eye disorders and thus they have been classified in group 3

A more detailed study of the children treated from 4 years of age is in progress Subjective symptoms caused by heterophoria are rare in this age group When present however they are an indication for treatment and therefore they have here been considered as significant health problems

Different screening levels of the visual acuity will influence the proportions of significant and non significant eye disorders referred for diagnosis and treatment The consequences of different screening levels in the present study are evident from Fig 1 and Table 3 A lowering of the passing standard from 1 0 to 0 9 would reduce the referral of children with non significant disorders from 75 5% (77/102) to 70 4% (50/71) At the same time 16% of all children needing treatment would have been overlooked (4/25)

In the same way, a passing standard of 0 7 would mean a reduction to 41% (9/22) of th

non significant cases and an increase of the overlooked cases to 48% (12/25)

In selecting cut off levels for referring children from an eye screening program both medical and administrative aspects must be taken into consideration and thus an analysis like the above mentioned is a necessary step in the critical checking of the program

If possible the initial time consuming and trying treatment of functional amblyopia and other significant eye disorders should be terminated when school starts (4 9) Also as a principle the visual prognosis is better if the treatment is instituted early (1 6 8) Therefore it is an important achievement of the preschool vision screening program when it is demonstrated as in Table 4 that the risk of finding a new significant eye disorder in a school entrant is more than 6 times greater for a child who was not examined in his preschool years and the risk of finding an amblyopic child is more than 10 times greater

These figures are based on the assumption that no visual changes occurred in the populations between 4 and 7 years of age However it is quite conceivable that some significant eye disorders particularly myopia may become apparent during this time (3) If cases of myopia are eliminated from the newly detected significant eye disorders in Table 4 the difference between the two populations will be even greater (0 4% previously eye screened vs 3 7% previously not eye screened)

Thus it can be concluded that vision screening of preschool children is an important item and should be included in the regular child health services

ACKNOWLEDGEMENT

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CASE REPORT

SPLENOGONADAL FUSION

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ABSTRACT Halvorsen J F and Stray O (Department of Surgery Haukeland Hospital University of Bergen Bergen Norway) Splenogonadal fusion Case report Acta Paediatr Scand 67 379 1978 —A case of splenogonadal fusion of the continuous type occurring in a 14-month-old boy with limb deformities is reported and the literature briefly reviewed The ectopic splenic tissue was extirpated and follow up 1 year later showed normal position and development of the left testicle

KEY WORDS Splenogonadal fusion

Splenogonadal fusion is a rare enough congenital anomaly to warrant reporting Since Bostroem s first mention of the anomaly in 1883 (1) and Pommer s first detailed description in 1889 (8) an additional 70 cases have been reported in the literature In most cases the preoperative—as well as the operative—diagnosis was incorrect due to lack of knowledge of the condition

The present patient was admitted to the Department of Surgery under the diagnosis of torsion of the testicle which was also the preoperative diagnosis

CASE HISTORY

The patient a 14-month-old boy had since birth been under orthopaed c treatment for congenital malformations of the limbs Three days prior to admission the mother noted a swelling of the left scrotum During the following days the child was irritable He was admitted to the Department of Surgery on May 6 1975 with a diagnosis of torsion of the testicle He had deformities of three limbs The left upper extremity was hypoplastic with fusion of the fingers The left lower limb was hypoplastic and there was no forefoot The right lower extremity was hemimelt The right upper limb was apparently normal In the left scrotum a [illegible]×2×3 cm large tumour was found and a thickened cord like structure was palpated in the left inguinal region A preoperative diagnosis of torsion of the left testicle was made and shortly after admission an exploratory incision in the left scrotal and inguinal region was performed under general anaesthesia A patent peritoneal processus vaginalis was found inside which a cord of brownish tissue with a diameter of 4 mm was found Distally the cord broadened and fused with the upper pole of the testicle (Fig 1) The testicle and epididymis were normal Proximally the cord entered the abdominal cavity through the internal inguinal ring The abdomen was opened through a lower left gridiron incision The cord of brownish tissue was found to cross the transverse colon anteriorly slightly proximal [redacted] the splenic flexure and fuse with the lower pole of the spleen The cord was resected proximally at its narrowest point cm below the spleen and distally through the transition zone of fibrous tissue connecting it with the upper pole of the testicle The hernial sac was extirpated and the incisions closed Histological examination showed normal splenic tissue In the transition zone small areas of infantile testicular tissue were found The postoperative course was uneventful and follow up a year later showed normal development and position of the left testicle

DISCUSSION

In 1974 Mizutani et al (5) stated that 68 cases of splenogonadal fusion had been reported in the literature including their own case Since

abdomen and in the scrotum when treating hypersplenism and other blood dyscrasias with splenectomy (6) Spleen scanning is a helpful tool in locating ectopic splenic tissue (2)

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Fig 1 Operative findings Asterisk indicates testicle and triangle shows ectopic splenic tissue

then an additional 4 cases have been reported (2 4 7 13) bringing the total to 72 The condition has occurred almost exclusively in the male and only 4 female cases have been recorded (see 7 for references) The left gonad has been involved in all but one case (see 7 for reference)

The anomaly has been described in the literature under various headings ectopic or accessory spleen in the scrotum caudate spleen and splenogonadal fusion From the embryological point of view the latter term is probably the most informative The origin of the malformation may be dated to the period when the splenic anlage is in close proximity with the mesonephros and the gonadal anlage i e between the 4th and 8th weeks of embryonic life The frequent association of limb malformations and micrognathia with the continuous type of splenogonadal fusion also points to the same period as the limbs and mandible begin to differentiate at 6–7 weeks of gestational life This seems to indicate that something diffuse and non fatal has occurred in the embryo around the 6th or 7th week of intra uterine life (9)

Putschar & Manion (9) classified the anomaly into two different forms In the continuous type a cord of splenic or fibrous tissue connects the spleen and the gonad and it is frequently associated with limb deformities (amelia hemimelia peromelia phocomelia) and micrognathia less frequently it is associated with a variety of other malformations such as anal atresia asymmetry of the skull and abnormal fissures of the lungs and liver (9) In the discontinuous type one or more masses of accessory splenic tissue is attached to the gonad but there is no connecting cord between this and the spleen In none of these have associated anomalies been reported

In nearly all the cases the anomaly represented an unexpected finding at operations performed under the incorrect diagnoses of incarcerated inguinal hernia testicular tumour torsion of the testicle third testicle epididymo orchitis thrombosis of the spermatic vessels Sometimes it was an incidental finding at operations for unrelated causes and at autopsy One case has been reported in which the cord connecting the spleen with the testicle caused external obstruction of the transverse colon (3) Another case with traumatic rupture of the ectopic splenic tissue was described by Sforza (10) Cases with pain and scrotal swelling during attacks of malaria are on record (11 12) In only 1 case (2) was a correct diagnosis made not at operation using spleen scanning in a 14 month old black boy with limb deformities and an undescended left testicle

These considerations have important implications for both diagnosis and treatment the anomaly should enter into consideration in the diagnosis of a left sided scrotal mass or cryptorchism especially when these conditions are associated with limb deformities and or micrognathia In the past many normal testicles have been unnecessarily sacrificed due to lack of knowledge of the nature of the scrotal mass when extirpation of the ectopic tissue would have been sufficient Another important implication is that a search should be made for ectopic splenic tissue both in the

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CASE REPORT

ABNORMAL NEUTROPHIL CHEMOTAXIS IN A SYNDROME OF UNUSUAL FACIES PROPORTIONATE SMALL STATURE AND SENSORINEURAL DEAFNESS MUTISM

Y H THONG B S DOUGLAS and A FERRANTE

From the Department of Paediatrics University of Adelaide and the Department of Surgery Adelaide Children s Hospital Adelaide Australia

ABSTRACT Thong Y H Douglas B S and Ferrante A (Department of Paediatrics and Department of Surgery Adelaide Children s Hospital North Adelaide South Australia Australia) Abnormal neutrophil chemotaxis in a syndrome of unusual facies proportionate small stature and sensorineural deafness mutism Acta Paediatr Scand 67 383 1978.—Two of 5 children in one family presented with unique facies proportionate small stature and sensorineural deafness mutism One of the children who had a history of recurrent infections was shown to have a defect of leukocyte chemotaxis Although impairment of chemotaxis could not be demonstrated in the other affected sibling it is unlikely that the association of a previously undescribed syndrome and a rare disorder of chemotaxis is a chance occurrence

KEY WORDS Abnormal chemotaxis unique facies proportionate short stature sensorineural deafness

Primary disorders of chemotaxis are uncommon (26) A history of recurrent infections in a child with an unique facies proportionate small stature and sensorineural deafness mutism led to the discovery of a leukocyte chemotactic defect One brother who shared her unusual features did not show a defect in chemotaxis The association between two rare disorders such as a previously undescribed syndrome and a neutrophil chemotactic defect would seem to us more than a chance occurrence

CASE REPORT

A 3 month-old Caucasian girl (M J K) was admitted to the Adelaide Children s Hospital for investigation of immune function She had a history of pneumonia multiple episodes of purulent otitis media and most recently a submandibular abscess from which *Staphylococcus aureus* was cultured This abscess required surgical drainage on three occasions and took 6 weeks to heal despite appropriate antibiotic therapy

The birth was premature at 37 weeks gestation Birth weight was 1984 g Turricephaly was noticed at birth and the underlying coronal synostosis surgically reconstructed at 2 months of age Immunizations with DPT oral poliomyelitis and live measles vaccines were well tolerated

Physical examination (Figs 1*a* 2*a*) revealed a small girl with narrow face long nose receding mandible and high arched palate The orbits were small with hypertelorism and antimongoloid slant of the eyes the ears were small and low set The height was 75 0 cm (<3%) weight 7 5 kg (<3%) head circumference 45 2 cm (10%) arm span 71 5 cm and height minus arm span −3 5 cm (average for age) Psychomotor development was appropriate for age except in the area of language development and social behaviour as a result of sensorineural deafness An anterior ectopic anus was also found

Laboratory investigations showed normal peripheral

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Fig 3 Radiograph of lower limbs of M J K at age 18 months showing coarse trabecular pattern and splayed epiphysis



Fig 4 Lateral X ray view of skull of M J K at 12 months of age showing shallow posterior fossa

obtained by using a grid on the eyepiece of a Nikon inverted microscope Chemotaxis (A) and random mobility (B) are expressed in mm/ hr

RESULTS

M J K

Her serum concentration of IgG was 924 mg/dl IgM 75 mg/dl IgA 133 mg/dl IgE <6 IU/ml C3 116 μg/ml C4 42 μg/ml and CH50 132 U/ml The E rosettes were 61% and B cells 25% Response of lymphocytes to PHA was 22 994/1 872 cpm ^{3}H thymidine uptake of triplicate samples Random tetanus antibody titre was 1 32 Hexose monophosphate shunt and bactericidal activity of her neutrophils were normal In contrast both random mobility and chemotactic responses tested on multiple occasions over 3 months were impaired compared with controls (Fig 5) The random mobility of her neutrophils was 0 21 ± 0 03 mm/2 hr (mean ± S D) while controls were 0 32±0 11 mm/2 hr ($p<0\ 01$) Her neutrophil chemotactic response was 0 71±0 15 mm/2 hr compared with 1 37±0 14 mm/2 hr in controls ($p<0\ 001$) Her serum at concentrations of up to 20% did not inhibit chemotaxis of her own neutrophils or control neutrophils

In vivo chemotaxis was also impaired Coverslips removed at 2 4 and 8 hour periods showed <10 PMN per coverslip Using the scoring system of Gewurz et al (7) the total score was 3+ a score of <6+ indicates impaired response

However neutrophil marrow and marginal pools were both adequate Peripheral neutrophil count rose from 3 840/μl to 7 700/μl 5 hours after i v hydrocortisone and rose from 4 526/μl to 10 152/μl 1 hour after subcutaneous



Fig 1 Full frontal view of face of patient M J K (*a*) and her affected brother S K (*b*) S K was wearing a wig because of alopecia caused by cancer chemotherapy

blood count urinalysis and serum concentrations of electrolytes glucose urea calcium magnesium phosphorus alkaline phosphatase thyroxine growth hormone and somatomedin Chromosomal analysis showed a normal female karyotype

Radiographs of long bones showed a generalized reduction of bone density with a coarse trabecular pattern and splayed epiphysis (Fig 3) The skull was high and short with a small posterior cranial fossa (Fig 4) Intravenous pyelogram was normal

Family and Affected Brother

Except for her brother none of the other family members including 3 siblings (males aged 11 and 16 years and a female aged 8 years) shared the unusual features Father s age was 36 years and mother s age 33 years There was no parental consanguinity

S K her 10-year old brother (Figs 1*b* 2*b*) shared the unique facies proportionate small stature deaf mutism and normal intelligence Height was 115 cm (<3%) weight 16 8 kg (<3%) His birth was also premature at 36 weeks gestation with a birth weight of 1 780 g However cranial synostosis and ectopic anus were absent He suffered several episodes of otitis media and one attack of penumonia in infancy Proneness to infection was not evident during childhood Measles and chickenpox were well tolerated Extensive studies done at 8 years of age for small stature including metabolic hormonal and chromosomal determinations revealed no abnormalities Orchidopexy was performed for bilateral undescended testes at 9 years of age He developed an osteosarcoma of the right proximal humerus soon after which was treated by surgical excision and chemotherapy At the time of writing he has an osteomyelitis at the site of cancer surgery and continues to receive antibiotic chemotherapy Biopsy did not show recurrence of the tumour

MATERIALS AND METHODS

Immune function tests were done using standard tech niques Serum immunoglobulins (A G M) and comp ment (C3 C4) were measured by radial immunodiffus using commercial plates (Behringwerke W German serum IgE by the Phadebas IgE test kit (Pharmac Sweden) serum total hemolytic complement by the ly of sheep red cells (15) T-cells by the E rosette te (20) B cells by fluorescent staining of surface immun globulins (13) lymphocyte transformation to PHA by micro-assay procedure (23) neutrophil bactericidal c pacity by the method of Holmes et al (11) and hexos monophosphate shunt activity by the conversion of ^{14}C 1 glucose to $^{14}CO_2$ (24)

The adequacy of the marrow neutrophil reserve w measured by obtaining a total white and differential cou by fingerprick at 0 and 5 hours after intravenous inje tion of 50 mg hydrocortisone (6) The adequacy of th marginal neutrophil pool was assessed by total white a differential counts before and 1 hour after the su cutaneous injection of 0 07 ml 1 1000 solution of e nephrine (19) In vivo chemotaxis was examined by th Rebuck skin window technique (7)

In vitro chemotaxis was performed by the agarose tec nique (12 18) with slight modifications (25) Brief agarose gel plates were prepared by mixing 3 ml of agarose solution with 3 ml medium 199 containing 10 heat inactivated fetal calf serum Three wells of 2.5 m diameter were cut on a straight axis 2 3 mm apa measured edge to edge with the help of a template the centre well was added 4 µl of medium 199 contain 2×10^5 neutrophils purified by Hypaque–Ficoll centrifug tion (4) To the outer well was added 2 µl of chem tactic stimulus generated by incubating 1×10^9 yeast ce of Torulopsis glabrata with 0 5 ml pooled human ser for 30 min at 37°C The inner well received 2 µl mediu only The plates were incubated at 37°C for 2 hours in 5% CO_2 atmosphere and high humidity after which t distance travelled by the 10 fastest moving neutroph toward the outer well (A) and the inner well (B) w



Fig 2 Lateral view of face of M J K (*a*) and S K (*b*)

syndrome appears to be the result of a mutation affecting autosomal genes and neutrophil chemotactic function may be controlled by closely linked genes

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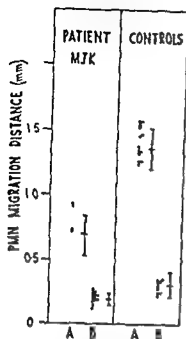


Fig 5 Neutrophil chemotaxis and random mobility of M J K tested on multiple occasions when she was well and free from infection

epinephrine There was also excellent neutrophil response to infection

S K

His serum concentration of IgG was 913 mg/dl IgM 116 mg/dl IgA 55 mg/dl and IgE <6 U/ml E rosettes were 41% and B cells 18% Response of lymphocytes to PHA was 8675/672 cpm ^{3}H thymidine uptake of triplicate samples Bactericidal activity of neutrophils was normal

In vitro random mobility and chemotaxis of S K , as well as the rest of the family were within normal limits His random mobility was 0 5 mm/2 hr and chemotaxis was 1 5 mm/2 hr

DISCUSSION

The peculiar facies proportionate small stature and deaf mutism in these 2 siblings is a unique combination of features suggestive of a new syndrome Only superficial resemblances can be found to the Seckel syndrome (22) and the Hallerman Strieff syndrome (27) Immuno deficiency has been described in association with short limbed dwarfism (1) but the defects are confined to antibody and/or cell mediated immunity

Neutrophils constitute an important mechanism of host resistance to infection (?) Chemotaxis or the ability of PMNs to promptly reach the site of infection may be crucial to the outcome of an infection Susceptibility to infection is the hallmark of the chemotactic disorders that have been described (5 9 10 11 18 19) The increased susceptibility to infection in M J K may be ascribed to a defect of neutrophil chemotaxis demonstrable both in vivo and in vitro In vitro tests on multiple occasions over a 3 month period during time when she was well and free from infection consistently showed poor neutrophil chemotactic responsiveness

The findings in her brother S K are less clear His clinical problems prevented us from studying his immune function thoroughly At the time of study he had received cancer chemotherapy for a period of one year The depressed response of his lymphocytes to PHA and the lower percentage of E rosettes may reflect this In vitro chemotaxis performed when he had not been receiving cytotoxic drugs for 1 month showed a normal response It must be assumed that he does not have a chemotactic defect His proneness to infection during infancy was not evident during childhood Of some pertinence to this discussion is the report by Blum et al (3) of divergent chemotactic responses in a father and son with the syndrome of recurrent infections hyperimmunoglobulinaemia E and eczema

Besides chemotaxis other incongruent features between brother and sister include the presence of coronal synostosis and ectopic anus in M J K but not her brother The question arises whether the association between this previously undescribed syndrome and the chemotactic disorder is purely coincidental Others (14 21) have shown that chromosomal disorders may be associated with defective neutrophil movement This new

yndrome appears to be the result of a muta on affecting autosomal genes and neutrophil hemotactic function may be controlled by losely linked genes

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CASE REPORT

CLINICAL, HORMONAL AND ULTRASTRUCTURE STUDIES OF A VIRILIZING HEPATOBLASTOMA

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ABSTRACT Kumar E V Kumar L Pathak I C Dash R J and Joshi V V (Departments of Pediatrics Pediatric Surgery Endocrinology and Pathology Postgraduate Institute of Medical Education & Research Chandigarh India) Clinical hormonal and ultrastructure studies of a virilizing hepatoblastoma Acta Paediatr Scand 67 389 1978 —Virilizing hepatoblastoma was found to be the cause of precocious puberty in a 3½ year-old boy Both human chorionic gonadotropins and alphafetoprotein in increased amounts were detected in serum and tumor tissue These substances disappeared from the blood after removal of the tumor Ultrastructure studies revealed the presence of dense membrane bound secretory granules responsible for the hormone production

KEY WORDS Hepatoblastoma precocious puberty

Hepatoblastoma is a rare cause of precocious puberty To date only 12 such cases have been reported (1–3 5 9 10 12 14 16 19–21) The tumor tissue elaborates a substance similar to human chorionic gonadotropin (hCG) (3 10) and this stimulates testosterone production leading to isosexual precocity The tumor also secretes alphafetoprotein (αFP) in large amounts (10) Detection of these circulating substances is of practical importance for early diagnosis and evaluation of treatment (3)

We describe a case of precocious puberty in a 3½ year old boy with a hepatoblastoma High titres of hCG and αFP were demonstrated in serum and tumor tissue The ultrastructure studies of this hormone secreting hepatoblastoma are also described

CASE REPORT

P K a 3½ year-old male child was admitted for investigation of precocious puberty His development was normal until 6 months before admission when a rapid increase in height and deepening of his voice were noted There was also rapid enlargement of the penis and growth of pubic hair Frequent penile erections without ejaculation had occurred Liver enlargement had been first noted at the age of 6 months

His height was 100 cm weight 16 5 kg blood pressure 100/70 mmHg and pulse rate 98/min The ophthalmic *fundus* was normal His voice was deep He had dark pubic hair as well as sparse facial and axillary hairs The penis measured 6 5 cm in length The scrotal skin was pigmented Both testes were symmetrically and uniformly enlarged measuring 3×2×2 cm each The prostate was palpable on rectal examination The liver was enlarged 6 cm below the right costal margin its surface was smooth and firm and its margin rounded The spleen was palpable 4 cm below the left costal margin There was no ascites

Laboratory data

The haemoglobin was 5 33 mmol/l Erc were normocytic and normochromic Total serum protein was 65 g/l with albumin 42 g/l and globulin 23 g/l The serum bilirubin was 4 77 μmol/l The prothrombin time index was 100% SGOT was 0 57 μmol s⁻¹/l and SGPT 0 23 μmol s⁻¹/l Serum alkaline phosphatase was 8 65 μmol s⁻¹/l There was no appreciable change in these values 2 weeks after operation An intravenous pyelogram E E G X ray of the chest and X rays of skeletal survey were normal The bone age was between 5 and 6 years X ray of the abdomen showed a small area of calcification within the

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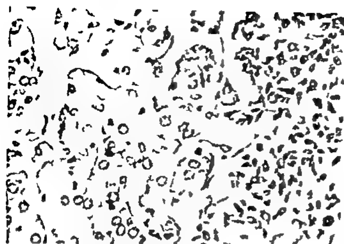


Fig 1 Photomicrograph of hepatoblastoma showing two morphologic cell types small cells with darkly staining nuclei scanty cytoplasm arranged in loose clusters and larger cells with vesicular nuclei moderate amount of cytoplasm resembling liver cells Hematoxylin and eosin ×400

liver The inferior venacavogram showed tumor flush in the arterial phase Hepatic scan using intravenous injection of a sulphur colloid 99 m Technetium was normal Urinary 17 ketosteroid excretion (17) was 34 67 µmol/l Serum αFP (22) was more than 1 µg/ml hCG (4 18) was 430 mIU/ml LH 62 int U/l and FSH 0 9 mIU/ml Testicular biopsy showed moderate interstitial cell hyperplasia with no spermatogenesis A diagnosis of virilizing hepatoblastoma was made and the patient was operated upon There was a dusky red globular tumor 4 cm in diameter bulging from the postero-inferior surface of the right lobe of the liver Posteriorly it was adherent to the dome of the diaphragm The tumor was well encapsulated and could be completely resected along with a part of the diaphragm The right adrenal gland could not be located αFP was found in concentration of 80 µg/g in tumor tissue and hCG was 2300 mIU/g of tumor tissue Serum αFP and hCG were not detected twenty days after operation The tumor was lobulated and soft in consistency An area of 1 5 cm×1 cm was necrosed and calcification was present Microscopic examination showed that the tumor consisted predominantly of two cell types small cuboidal primitive cells with basophilic cytoplasm and larger polygonal cells with pale eosinophilic cytoplasm resembling normal liver cells closely (Fig 1) Occasional foci of squamous differentiation were seen In some areas tumor cells showed diastase resistant PAS positive material in the cytoplasm Besides these epithelial elements mesenchymal elements viz osteoid and bony spicules were seen Microscopic foci of vascular and capsular invasion were noted (Fig 2) Two lymph nodes from the porta hepatis did not show any evidence of metastasis Ultrastructural studies of the tumor tissue showed two types of tumor cells some with well developed and other with poorly developed organelles Bile canaliculi were identified Round membrane bound electron dense secretory granules varying from 100 nm to 160 nm were seen in the tumor cells (Fig 3)

In the postoperative period the size of the liver and spleen remained unaltered Vincristine 0 07 mg/kg once weekly (3 doses) and methotrexate 0 25 mg/kg daily for 3 weeks were given The size of the penis decreased to 4 5 cm by the 40th postoperative day The child developed ascites and further enlargement of the liver There was no radiological evidence of metastases in the lungs The patient was discharged from hospital at his family's request and died 1 month later

DISCUSSION

Virilizing hepatoblastoma is a rare malignant tumor and results in isosexual precocity due to high circulating levels of testicular androgens in response to ICSH (14) or hCG (3) secreted by the tumor tissue The presence of significant hepatomegaly in a child with isosexual precocity is characteristic (1–3 9 10 12 14 19 21) and should alert a clinician to suspect this syndrome It is generally seen in early childhood (14) Penile and testicular enlargement as seen in our patient has been reported in most of the children (1–3 9 10 12 16 19–21) Metastases to lungs (1–2 9 10 12 19–21) bones (2 10 20) abdominal lymph nodes (9 21) the other lobe of the liver (14) and the brain (1) have been observed We could not detect any metastases by clinical or radiological observation although venous in



Fig 2 Photomicrograph of hepatoblastoma showing vascular invasion Note that the tumor tissue is adherent to the wall and protruding into the lumen of a venous channel Hematoxylin and eosin ×110

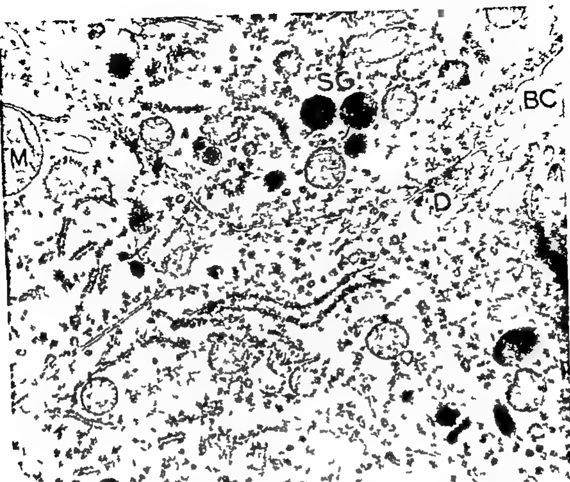


Fig 3 Electron micrograph of hepatoblastoma showing parts of three tumor cells Note the electron dense round membrane bound secretory granules (*SG*) in one of the cells Desmosomes (*D*) are present On the right side a bile canaliculus (*BC*) with microvilli of tumor cells projecting into it is seen Scattered rough endoplasmic reticulum and mitochondria are also seen Original magnification ×15000 Actual magnification ×64000 Uranyl acetate and lead citrate

vasion was evident on microscopic examination of the tumor Malignant liver tumors in childhood are associated with an increased incidence of congenital anomalies (7) Virilizing hepatoblastomas are no exception (10 14 16) In the case being reported the right adrenal gland was absent Calcification of tumor tissue as seen in this patient was also observed in 2 other cases (9 14) Histological examination of the testes revealed interstitial cell hyperplasia with no spermatogenesis Such findings were present in most instances (2 3 5 10 12 14 19 21)

Assay of gonadotropins from the extracts of tumor tissue has been done only in 4 cases (3 10 14 19) Chorionic gonadotropin activity was seen in one (19) In another although no hCG could be detected in tumor extract the circulating levels of hCG were high This suggests active secretion of this hormone by the tumor tissue (10) In a third case administration of tumor extract preincubated with hCG antiserum increased the weight of hypophysectomised rat prostate thus suggesting similarity of the tumor hormone to interstitial cell stimulating hormone (14) In the fourth case the tumor tissue culture produced both hCG and αFP (3) Using a highly specific radioim-

munoassay for hCG we have demonstrated the presence of this hormone in both tumor extract and serum We have also demonstrated αFP in the tumor extract These substances could not be detected on the 20th postoperative day The secretory function of these tumors can be suppressed by chemotherapy though the tumor may continue to grow and even metastasize (3 10) The experience in adults with hepatomas producing αFP (13) has been similar

The hepatocytic origin of the tumor is confirmed by the ultrastructural demonstration of bile canaliculi with microvilli on the tumor cell surface (Fig 3) Another notable ultrastructural feature which has not been previously reported was the presence of round membrane bound electron dense granules in the tumor cells (Fig 3) Secretory granules of similar cytochemical nature (i e diastase resistant PAS positive) and electron microscopic appearance have been demonstrated in the gonadotropic cells of the anterior pituitary (6) and in the syncytiotrophoblast of human placenta (23) In our case hCG was isolated from the tumor tissue Therefore it appears that the secretory granules seen in the tumor cells are hCG or its precursor Electronmicroscopic studies of hepatoblastoma previously reported in the literature have not shown secretory granules in the tumor cells However these patients did not have any endocrine syndromes (8 11 15)

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REVIEW ARTICLE

FREE AMINO ACID DIETS IN THE VICIOUS CIRCLE OF DIARRHOEA–MALNUTRITION–MALABSORPTION DURING INFANCY

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The vicious circle of protein-energy deficiency and diarrhoea involves low intake defective utilization of alimentary proteins and sugar through reduced pancreatic enzyme secretion (2) and atrophy of surface jejunal and colonic epithelium (7 27 32) with reduced dipeptide hydrolase (6 19) and disaccharidase activity (3 16) It also involves impaired reabsorption of endogenous nitrogen amino acids (8) and fat (33) An altered duodenal (22) and jejunal (1?) bacterial flora with a net secretion of fluid into the jejunum (17) and impaired synthesis of intestinal mucosa (4) lead to a stage of intractable diarrhoea The relative importance of acute infections or chronic bacterial proliferation and protein-energy depletion itself has not been determined

Good results have been achieved by the use of total intravenous feeding (15) to interrupt this vicious circle However total intravenous feeding should at present be restricted to centres which have all the resources required to deal with the necessary biochemical monitoring the complications of the technique (11) and the exploration of the hitherto largely unknown metabolic and neurotoxic consequences of intravenous amino acid administration to infants (23 29) Moreover as studies in semi starved rats and guinea pigs suggest an increased absorption of amino acids and glucose in malnutrition (9 13 30) it seems that the possibility of peroral amino acid and monosaccharide feeding (elemental diet) should be more extensively investigated Such diets also eliminate the theoretical risk of an abnormal transfer of intact protein molecules across the damaged mucosa with a risk of inducing a food allergy This risk seems to be a reality as protein deficient rats have increased pinocytosis later deterioration of apical junctions and movement of protein molecules directly between mucosal cells (38)

Free amino acid diets as developed and investigated by Greenstein et al (10) are known to be absorbed at a slower rate than protein hydrolysates (28) but have the advantage of a flexible and reproducible composition and may be administered in a more ideal composition corresponding to the known requirements of infants This diet also eliminates the risk of abnormal peptide transfer A disadvantage however is high osmolality with a risk of complicating osmotic diarrhoea On the other hand the diet can be packed in sealed packages as a water soluble powder with good storage stability and provide maximum safety when handled under primitive conditions

The advantages of an elemental diet in the treatment of the short gut syndrome with diarrhoea and undernutrition of adults have been reported (34) However amino acid diets have been reported to give rise to haemolytic anaemia and pancreatic acinar atrophy and fibrosis in rats (21) This may be a consequence of

malnutrition or the change in specific indigenous microflora (36) during prolonged treatment and such adverse effects have not been seen upon the short administration of similar diets to man (26) Amino acid based diets have also been used for extended periods in the dietary treatment of inborn errors of amino acid metabolism predominantly in phenylketonuria without such complications

In a recent investigation (24) using a commercially available elemental diet it seemed that we were unable to provide sufficient amino acids to the peripheral blood to keep pace with the high supply of glucose However it should be considered that the perorally administered amino acids could be partially used for local mucosal repair and in this way peroral treatment could be a necessary part of the early rehabilitational procedure Investigations on semi starved rats with mucosal atrophy have shown an increased mucosal incorporation of radioactive leucine (14) The incorporation was significantly higher into the proximal part of the small intestine than into the distal part This was seen when amino acids were given perorally but not parenterally

It is important to realize that by the use of elemental diets we impose an unphysiological diet where the normal intricate mechanisms for providing the different amino acids in optimal concentration simultaneously to the body tissues may be disturbed There may be a correlation between how rapidly certain amino acids are released from proteins during digestion and their transport rates Ideally an effective elemental diet should be composed of a partial hydrolysate of protein containing small peptides in addition to amino acids Amino acids with a particularly slow absorption rate in the free state seem to be absorbed faster from an enzymic hydrolysate in which they occur as peptides The different transport channels surface hydrolysis of peptides followed by amino acid uptake and peptide transport followed by intracellular hydrolysis (5) explain the lower competition for transport using peptides rather than amino acids in intestinal intubation experiments (28)

At present however the risk of pathological entry of intact peptides into the blood cannot be estimated This possible complication of peroral feeding with partial hydrolysates should be taken into account But recent experiments in rats (1) have shown that intravenous administration of dipeptides is as effective as that of the corresponding free amino acids in enriching the tissue pools of amino acids suggesting an efficient hydrolysis by cellular enzymes prohibiting the accumulation of intact dipeptides in body tissues It therefore seems that it could be justified to use controlled partial hydrolysates containing known amounts of small peptides especially as this would reduce the osmolality of the diet A peptide solution prepared from synthetic dipeptides may prove to be too expensive for clinical use and our knowledge concerning peptide transport is as yet insufficient to compose an ideal dipeptide mixture

At present the alternative of administering free amino acids with a composition simulating human milk protein but with increased concentrations of those amino acids known to have a low absorption rate seems to be preferable The balance between the amino acids of the different transport systems must be kept in mind when composing an elemental diet as well as the interactions between basic and neutral amino acids (25) in order to eliminate as far as possible undue inhibition of transport This is most important in relation to the concentrations of the amino acids known to have a very high affinity to the carrier The fact that the acidic amino acids and glutamine are partially metabolized in the small intestinal tissues (35 37) should be remembered when dealing with the problem of the neutrality and the metabolic effect of the diet

A recent rehabilitation study of marasmic infants with a diet lacking cystine (24) gave very low plasma cystine concentrations indicating that this amino acid is essential to the

starving infant and should be included in the diet This could theoretically be due to a de layed cystathionase induction (31) and/or vita min B deficiency The need for folate vitamin B_6 and B_1 in excess of basic needs should be accounted for as amino acid transport and metabolism requite an extra supply and as there may be both a subclinical deficiency and malabsorption (18) of vitamins after pro longed starvation

In addition to the suggestions above con cerning the amino acid composition of the re habilitational diet it should be emphasized that the elemental diets available are not adapted to the special requirements of infants partic ulary regarding salt (usually too high espe cially relevant in dealing with undernourished individuals with salt retention) and fatty acid content (especially those containing MCT and not providing sufficient amounts of essential fatty acids) However this may easily be cor rected by reference to already available infor mation The alternative of using glucose poly mers instead of monosaccharides in order to further reduce the osmolality of the diet (20) should also be tried

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malnutrition or the change in specific indigenous microflora (36) during prolonged treatment and such adverse effects have not been seen upon the short administration of similar diets to man (26) Amino acid based diets have also been used for extended periods in the dietary treatment of inborn errors of amino acid metabolism predominantly in phenyl ketonuria without such complications

In a recent investigation (24) using a commercially available elemental diet it seemed that we were unable to provide sufficient amino acids to the peripheral blood to keep pace with the high supply of glucose However it should be considered that the perorally administered amino acids could be partially used for local mucosal repair and in this way peroral treatment could be a necessary part of the early rehabilitational procedure Investigations on semi starved rats with mucosal atrophy have shown an increased mucosal incorporation of radioactive leucine (14) The incorporation was significantly higher into the proximal part of the small intestine than into the distal part This was seen when amino acids were given perorally but not parenterally

It is important to realize that by the use of elemental diets we impose an unphysiological diet where the normal intricate mechanisms for providing the different amino acids in optimal concentration simultaneously to the body tissues may be disturbed There may be a correlation between how rapidly certain amino acids are released from proteins during digestion and their transport rates Ideally an effective elemental diet should be composed of a partial hydrolysate of protein containing small peptides in addition to amino acids Amino acids with a particularly slow absorption rate in the free state seem to be absorbed faster from an enzymic hydrolysate in which they occur as peptides The different transport channels surface hydrolysis of peptides followed by amino acid uptake and peptide transport followed by intracellular hydrolysis (5) explain the lower competition for transport using peptides rather than amino acids in intestinal intubation experiments (28)

At present however the risk of pathological entry of intact peptides into the blood can not be estimated This possible complication of peroral feeding with partial hydrolysates should be taken into account But recent experiments in rats (1) have shown that intravenous administration of dipeptides is as effective as that of the corresponding free amino acids in enriching the tissue pools of amino acids suggesting an efficient hydrolysis by cellular enzymes prohibiting the accumulation of intact dipeptides in body tissues It therefore seems that it could be justified to use controlled partial hydrolysates containing known amounts of small peptides especially as this would reduce the osmolality of the diet A peptide solution prepared from synthetic dipeptides may prove to be too expensive for clinical use and our knowledge concerning peptide transport is as yet insufficient to compose an ideal dipeptide mixture

At present the alternative of administering free amino acids with a composition simulating human milk protein but with increased concentrations of those amino acids known to have a low absorption rate seems to be preferable The balance between the amino acids of the different transport systems must be kept in mind when composing an elemental diet as well as the interactions between basic and neutral amino acids (25) in order to eliminate as far as possible undue inhibition of transport This is most important in relation to the concentrations of the amino acids known to have a very high affinity to the carrier The fact that the acidic amino acids and glutamine are partially metabolized in the small intestinal tissues (35 37) should be remembered when dealing with the problem of the neutrality and the metabolic effect of the diet

A recent rehabilitation study of marasmic infants with a diet lacking cystine (24) gave very low plasma cystine concentrations indicating that this amino acid is essential to the

starving infant and should be included in the diet. This could theoretically be due to a delayed cystathionase induction (31) and/or vitamin B deficiency. The need for folate, vitamin B_6 and B_1 in excess of basic needs should be accounted for as amino acid transport and metabolism require an extra supply and as there may be both a subclinical deficiency and malabsorption (18) of vitamins after prolonged starvation.

In addition to the suggestions above concerning the amino acid composition of the rehabilitational diet it should be emphasized that the elemental diets available are not adapted to the special requirements of infants, particulary regarding salt (usually too high, especially relevant in dealing with undernourished individuals with salt retention) and fatty acid content (especially those containing MCT and not providing sufficient amounts of essential fatty acids). However, this may easily be corrected by reference to already available information. The alternative of using glucose polymers instead of monosaccharides in order to further reduce the osmolality of the diet (20) should also be tried.

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BOOK REVIEWS

W B Strong M Levy B Tomkins & M J Adams Jr *An introduction to pediatric cardiology* Charles C Thomas Publisher Springfield Illinois 1975 161 pp ill lus Price not given

The value of and the place for self instructing teaching aids can be discussed Medical teachers and students seem to be either pro or against The important thing is however that students learn in different ways and that some of them are becoming increasingly unhappy with the lecture system This book intends to offer an alternative to these students

The authors two paediatric cardiologists one general paediatrician and one expert in audiovisual education have cooperated to create a book that is a combination of current educational learning theory and modern practices in education

The book comprises 161 pages mostly figures and diagrams accompanied with short concise texts The book has five chapters The perinatal circulation general background of congenital heart defects common acyanotic congenital heart defects common cyanotic congenital heart defects and the large ventricular septal defect in infancy Each chapter starts with a statement of the objectives of that particular chapter then follows the actual text interfoliated by quiz questions and their answers It is understood that the student should have completed each chapter before he continues with the next one

The anatomy and the circulation both the normal and pathological in the various malformations dealt with is well described and richly illustrated with the so-called box diagram and with semischematic anatomical drawings The need to think haemodynamically is emphasized The students are taught the interrelationship between flow pressure and resistance and how these factors relate in nine of the most common congenital heart malformations covered by this book The pulmonary vascular changes occurring after birth and their implication in normal and pathological circulation are very well described and explained

One of the shortcomings of this book is in my opinion that very little or nothing is mentioned about clinical signs and physical and auscultatory findings While anatomy and haemodynamics are well documented one cannot help missing the correlation with physical findings and the clinical picture What is for example the haemodynamic correlate to the fixed splitting of the second sound and why is the murmur located over the pulmonary area in an atrial septal defect The last chapter on the large ventricular septal defect in infancy is more complete as the presentation offers a more clinical approach Here the above mentioned correlation between haemodynamics and clinical picture is at hand resulting in perhaps the best chapter in the book

The illustrations both diagrams and drawings some in colour are with few exceptions excellent The photographs are less outstanding

The book can be recommended as an *introduction* to paediatric cardiology for medical students nurses and physicians The book is also highly recommended to paediatric cardiologists involved in medical education A study of the book will no doubt give ideas of how to help students to learn paediatric cardiology

Bjorn Bjarke

J -C Larroche *Developmental pathology of the neonate* 545 pp illus Elsevier Excerpta Medica North Holland Amsterdam 1976 US $73 50 ISBN 90-?19 2107 3

This is a unique book It is the story of the personal experience of a paediatric pathologist working at a maternity hospital and an intensive care unit at the René Descartes University of Paris France

It is a book of courage the preface of which states that the author has left out the problems of teratology bone pathology genital abnormalities and inborn errors of metabolism It is courageous because it is an endeavor both to cover the entire subject and to be personal To cover the whole field of paediatric pathology in one book is impossible The vast field can no man—or woman—master so the book is very personal Thus in its in completeness it is very attractive very well illustrated and also authoritative The author tells us about what she knows It is significant that some 40% (00 pages) is devoted to paediatric neuropathology namely developmental aspects haemorrhagic lesions ischaemic changes Kernicterus encephalitis and CNS malformations

These chapters are particularly well written with many excellent illustrations and clinical correlations Only one relevant recent reference is missing i e Friede s Developmental Neuropathology of 1975

Several other important sections are large and well covered for example neonatal infections (47 pp) lungs (61 pp) urinary system (36 pp) adrenals (28 pp) Some aspects are more cursory e g immunologic problems (thymus 7 pp iso-imm 9 pp) oncology (occasional tumours outside the CNS) the placenta (14 pp) Dr Larroche is wise to refer the reader to already existing texts on these topics like we all have to in writing text books in paediatric pathology

Dr Larroche s volume is a very valuable book It should be on the reference shelf of all pathologists neonatologists and hospital paediatricians in general The publishers are again to be congratulated to the high quality of their printing standards

Biorn Ivemark

P P Kelalis L R King & A B Belman *Clinical pediatric urology* W B Saunders Co Philadelphia London Toronto 1976 2 volumes 1107 pp illus £50 – Vol 1 ISBN 0 7216 5350 2 Vol 2 ISBN 0 7216-5351 0

One of the latest contributions in the series of the text books from W B Saunders Company is devoted to urologic diseases in childhood The 29 sections written by 34 experts present a fairly complete survey in each area of clinical paediatric urology The text is lucid and the illustrations—including a large number of radiographs—are very good References are generously supplied and fairly up to date In the preface the authors state that the book has been written for the general urologist the paediatrician the resident and the interested medical student perhaps even to a greater degree than for the paediatric urologist The book deserves to be widely read The paediatrician may experience the large number of imaginative surgical methods and procedures as dead weight but will find many sections of particular interest as for instance Abdominal Masses (19 pp) Urinary Incontinence (22 pp) Obstructive Uropathy (97 pp) Vesico ureteral Reflux (?4 pp) Tumours and related disorders (104 pp) etc

Ludvig Okmian

Lula O Lubchenco *The high risk infant* Vol XIV *in* Major problems in clinical pediatrics (ed A J Schaffer & M Markowitz) 294 pp illus W B Saunders Company Philadelphia London Toronto 1976 £15 00 ISBN 0-7216-5800-8

This monograph in the series Major Problems in Clinical Pediatrics deals with the problems of defining the infant at risk of neonatal morbidity and later sequelae The author professor Lula O Lubchenco has more than 30 years experience in neonatal pediatrics In 1963 she contributed the first standards for intra uterine growth from the 24th to the 42nd gestational week and defined the concepts of AGA (appropriate for gestational age) LGA (large for gestational age) and SGA (small for gestational age) which have become basic parameters in all perinatal studies and in clinical pediatrics

In this volume she presents comprehensive data on incidence mortality morbidity and prognosis of high risk infants Although birthweight can be used to a certain extent to identify the infant at risk neonatal statistics show that infants with birthweights above 2 500 g constitute one third of the high risk population in most neonatal nurseries today The importance of gestational age or maturity of the newborn baby is pointed out and beautifully illustrated in this book with extensive chapters on the estimation of gestational age and on intra uterine growth and neonatal mortality and morbidity The pre term and post term infants the LGA and SGA infants the infants with congenital infections and the infants with congenital malformations are described in separate chapters This approach to the clinical problems shows the author's awareness and understanding of the needs of those in charge of newborn infants The final chapter on the long term outcome stresses among other things the importance of carefully controlled studies of new treatments

This book should be of great value not only for neonatologists but also for every pediatrician concerned with the general health care of children

Nils W Svenningsen

D M Ross & S A Ross *Hyperactivity Research theory and action* 385 pp John Wiley & Sons Ltd Chichester 1976 £13 75 ISBN 0-471 73678 3

The book gives a comprehensive review of the behaviour—or rather the behaviour pattern—that we have called hyperactivity The book commences with a historical section followed by chapters on etiology treatment research and practical advice The description is commendably balanced even though the chapter on psychotherapy shows that the authors have their main interest in behaviour therapy

In the opening chapters they stress that hyperactivity is a heterogeneous concept probably with a multifactorial background Genetic organic and environmental factors may all contribute It is regrettable that the distinction between the different kinds of hyperactivity is not consequently used when it comes to discussion of treatment and management Today there is much agreement that the treatment should depend on the kind of hyperactivity exhibited as well as on the etiology of the behaviour In one case a central nervous stimulant may be the drug of choice in another a drug with an opposite effect for instance a phenothiazine may be better On the other hand hyperactivity due to environmental factors may be better managed with some kind of psychotherapy But in most cases the background is multifactorial and consequently the therapy will also be multimodal

Considering their psychological training the authors treat the medical aspects of hyperactivity with great insight Not least the chapter on central nervous stimulants is readable and reasonable critical but not altogether negative On the contrary it is stated that drugs—including central nervous stimulants—have a place in the therapeutic arsenal although they should be used in a critical and restrained manner This chapter ought to be read by those who advocate a definite and complete prohibition of any use of central nervous stimulants for hyperactivity

One topic seldom covered is prevention Early detection may prevent the negative interaction between the hyperactive child and its environment This is one of the many responsibilities of child psychiatry and psychology within child health care The authors recommend the creation of a pediatric subspecialty in hyperactivity learning disorders and other school problems This recommendation must be due to an inadequate knowledge of the competence of child psychiatrists

The book also provides an extensive bibliography as well as methods of observation rating scales and measures On the whole this is a book which can be recommended to child psychiatrists pediatricians psychologists and others who want to extend their knowledge about the controversial concept hyperactivity

Torkel Scholander

K S Holt *Developmental paediatrics* Perspectives and practice In J Apley (ed) Postgraduate Paediatrics Series 311 pp illus Butterworths London Boston 1977 £5 50 ISBN 0-407-00065 8

An early argument for a separate medical specialty of paediatrics was that children are growing individuals and that the developmental aspect is so important that it influences not only the kinds of diseases that afflict children but also the course and prognosis of these diseases Since then many books about child development have been published some only considering the child from a neurological point of view others although very few trying to see the child and his development as the product of biological capacity and social circumstances

Kenneth Holt s book is of the first kind He writes clearly and comprehensively about the stages of maturation and development reflexes motor activities play activities language When reviewing theories of child development he states that a synthesis of the various views is the best way of rea hing a deeper understanding of the process It is very rare however to encounter sections where social aspects of development have been considered In the chapter on language development 3 lines are devoted to the influence of environmental factors although modern research (with its centre a few b ocks from Great Ormond Street) has taught us that the language and the need of language (and thereby also the development of language) differ between social classes In two chapters where typical' activities are described in children of various age groups horse riding is said to be typical at 9 years of age I would imagine that a large part of Bri ish children have not even seen a horse except on TV far less mounted one The pre reading phase is said to be up to 6 years of age In Sweden by definition this phase is up to 7 years because this is the age when children begin school

For a paediatrician starting his career or wanting to brush up his knowledge of normal and abnormal child development this book provides a good survey constructed logically and presented by an author with an immense experience of children The value of pure entertainment though is perhaps not so high the style is a bit dry and many photos small and insipid And it is a great pity that development as a neurological phenomenon is not supplemented with a broader recognition of social circumstances as restrictive and directive factors in the developmental process

Lennart Kohler

David W Smith *Recogni able patterns of human malformation* Genetic Embryologic and Clinical Aspects 2nd ed *In* J Schaffer (ed) Major Problems in Clinical Pediatrics vol VII 504 pp illus W B Saunders Company Philadelphia London Toronto 1976 US$15 50 ISBN 0-7216-8376-?

This is the second edition of a book that even with its first edition achieved great popularity among scientists and clinicians working with infants and children with congenital malformations

In the rapidly expanding field of malformation a constant updating is necessary and this new edition has been extended from 368 pages to 504 pages To the 135 syndromes described in the first edition several more have been added so that the book now includes [illegible] 5 descriptions

The major part of the book is devoted to short descriptions of different syndromes under separate headings Each presentation includes a description of the most frequent abnormalities found in that particular syndrome as well as its natural history and what is known about the etiology All descriptions are accompanied by illustrations of good quality and a few selected references for those who wish to read more At the end of the book there is also a valuable list of various anomalies which can constitute parts of different syndromes There is also a short chapter on minor malformations as a guide to the recognition of specific syndromes

In the second edition two new chapters have been added dealing with fundamental problems such as growth and mental deficiencies and the psychological adaptation to the child with a malformation These chapters are short and the contents are of varying quality Generally these questions are better dealt with in ordinary pediatric textbooks

This is a book that can be highly recommended as a catalogue for those working with malformed infants and children It is easy and pleasant to use due to the clear subgrouping of the syndromes It also gives a good background to the syndromes Those who want more information are provided with recent references

Marga eta Eriksson

ANNOUNCEMENTS

ELEVENTH ANNUAL MEETING OF THE EUROPEAN SOCIETY FOR PAEDIATRIC NEPHROLOGY

The 11th Annual meeting of the European Society for Paediatric Nephrology will take place in Kinat Anavim Israel on September 17–22 1978 For further information write the president Dr Harry Stark Beilinson Med Center Petah Tiqva Israel

CONGRESS OF PAEDIATRIC SURGERY

The Czechoslovak Paediatric Surgical Society arranges a congress of Paediatric Surgery with international participation Sept 26–29 1978 The subject will be neonatal surgery For further information contact the secre general Josef Koutecky M D CSc Klinika de chirurgie Ke Karlovu 2 12109 Praha 2 Czechoslova

SECOND INTERNATIONAL CHILD NEUROLOGY CONGRESS

The Second International Child Neurology Congress will be held in Sydney Australia November 26–30 1979 For further information write to the Congress Secreta Address GPO Box 3866 Sydney NSW 2001 Austr

3O VAHLQUIST
N MEMORIAM



[redacted] Vahlquist Co-Editor of *Acta Pædiatrica Scand* 1avica 1951–1976 and Chairman of the Board of Trustees of the Foundation of Acta Pædiatrica 965–1977 died March 31 1978 at the age of 69 years

Bo Vahlquist was one of the leading pediatricians of this century

After graduating from the Medical School of the University of Uppsala Bo Vahlquist commenced his training in pediatrics in Uppsala in 1938 He received his scientific degree in 1941 and was Associate Professor of Pediatrics 1946–1950 at the Karolinska Institutet under the chairmanship of Arvid Wallgren In 1950 he returned to his Alma Mater on being appointed to the Chair of Pediatrics in Uppsala In 1970 Bo Vahlquist resigned from this position to become advisor to the Swedish International Developmental Authority (SIDA) on matters related to nutrition

During his career Bo Vahlquist was entrusted with many important tasks in university and health administration in Sweden He was Dean of the Medical Faculty 1957–1960 and Pro-Rector of Uppsala University 1960–1962 During the years 1962–[redacted] he was member of the Swedish Medical Research Council and of the Board of the Swedish Agency for Research Cooperation (SAREC) since 1973

Bo Vahlquist was extremely active in international cooperation and collaboration On a number of occasions he served as a member of WHO Expert Committees on MCH and Nutrition and was from 1965 advisor to WHO on Maternal and Child Health problems and between 1968 and 1973 a member of the Protein Advisory Group of the UN He was also a member [redacted] the Executive Committee of the International Paediatric Association 1967–68 and of the Council of the International Union of Nutritional Sciences 1969–72

Bo Vahlquist started his scientific career even as a medical student After training in biochemistry he devoted his work to the field of pediatric hematology [redacted] which he soon became one of the pioneers Within a surprisingly short period of time he had made a number of important contributions among them his monograph on serum iron in infancy and childhood in normal and pathological conditions published as early as 1941 and which soon became a classic In Uppsala he was the leader of a very active group in pediatric hematology

During the years around 1950 Vahlquist and his co-workers made excellent studies on the transmission of antibodies from mother to fetus and on other factors of importance to the resistance to infections during early life

In 1959 Vahlquist and co-workers published a study on the clinical serological and biochemical findings on breastfed and artificially fed infants in Norrbotten County in Sweden Although the aim of the study which was to seek evidence of the superiority of breast milk to cow s milk formulas at a time when breast feeding was declining [redacted] Sweden and most other countries could not be fulfilled a number of contributions were made Another important aspect of this work was that [redacted] definitely stimulated Vahlquist to concentrate his further work on problems related [redacted] the nutrition of underprivileged children all over the world His reports on the results of studies on malnourished children in developing countries were followed by realistic plans for action as regards the organization of the

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In 1959 Vahlquist and co-workers published a study on the clinical serological and biochemical findings on breastfed and artificially fed infants in Norrbotten County in Sweden Although the aim of the study which was to seek evidence of the superiority of breast milk to cow s milk formulas at a time when breast feeding was declining in Sweden and most other countries could not be fulfilled a number of contributions were made Another important aspect of this work was that it definitely stimulated Vahlquist to concentrate his further work on problems related to the nutrition of underprivileged children all over the world His reports on the results of studies on malnourished children in developing countries were followed by realistic plans for action as regards the organization of the

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health service and of educational programs By combining a high degree of imagination with enormous working capacity Bo Vahlquist had always been able to make impressive achievements in all fields in which he has engaged himself The results of his research work on infant nutrition were followed by important practical applications leading to an improvement in the health situation of innumerable children all over the world

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Bo Vahlquist was greatly respected for his exemplary efficiency for his honesty and integrity and for his concern for underprivileged children the world over It was always his ambition to imp ment the results of his scientific work in the prac cal field and he was also very successful in tl respect

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Bo Vahlquist was a remarkable man and a colleague whose loss is deeply felt In his work he was driven by a constant and sincere concern for children in particular all the sick and hungry ones He was reticent about his inner feelings and deeper motives and only a few of his closest collaborators knew of his strong sympathy for suffering children They were also aware that this was the driving force behind his untiring efforts Insistently and forcibly he worked to improve the quality of children s lives His devotion his clear mind and his enormous working capacity made it possible for him to attain most of the goals he had set

He contributed greatly to the development of the present Swedish system of child health care and realised quite early the importance of subspecialisation within paediatrics He took a very active part in developing care and resources for handicapped children and from an early age medical education was a subject close to his heart

Throughout the years however research occupied most of his thoughts and time His approach was always comprehensive and often based on new ideas and hypotheses Within his major fields of interest—paediatric haematology immunology and nutrition—he made internationally recognised contributions from an early age By his example and often by an unparalleled helpfulness he furthered the research work of his younger colleagues many of them from abroad

From the early 1960s he became more and more passionately committed to the health problems of children in the Third World Again his organizing ability combined with his scientific approach led to the attainment of new knowledge and to important advances Undoubtedly his dedication in this field also gave added impetus to the Swedish aid to developing countries

Whenever Bo Vahlquist was asked to help and such requests were frequent he was always willing to comply and he invariably produced something worthwhile and useful Research writing and committee work were his main tools in promoting child health and he used these tools fervently until his very last days He is greatly missed by all his collaborators and by friends all over the world

Stig Sjolin

Acta Pædiatr Scand 67 403–411 1978

HOW TO APPROACH THE CHILD SUSPECTED OF MALABSORPTION

Experience from a Prospective Investigation of Suspected Malabsorption in Children 1968–1976 in Malmo

N O BERG S BORULF I JAKOBSSON and T LINDBERG

From the Departments of Paediatrics and Experimental Research Malmo General Hospital Malmo and Department of Pathology University of Lund Sweden

ABSTRACT Berg N O Borulf S Jakobsson I and Lindberg T (Departments of Paediatrics and Experimental Research Malmo General Hospital Malmo and Department of Pathology University of Lund Sweden) How to approach the child suspected of malabsorption Experience from a prospective investigation of suspected malabsorption in children 1968–1976 in Malmo Acta Paediatr Scand 67 403 1978 —180 children (mean age 20 months) suspected of malabsorption because of failure to thrive abnormal stools more than 3 weeks vomiting and/or abdominal distension were investigated with peroral small intestinal biopsy at duodeno-jejunal flexure (172 children) and/or duodenal intubation for analysis of trypsin and amylase activity in duodenal juice before and after a test meal of water (76 children) Results of xylose tolerance test lactose tolerance test faecal fat B-folate S-iron and S-albumin were related to morphology of mucosa A normal finding of one of these tests means in 15–26% a normal mucosa (diagnostic sensitivity) An abnormal finding means in 40–85% a severely damaged mucosa and in 85–100% a slightly moderately or severely damaged mucosa (diagnostic specificity) Combinations of these tests increase the diagnostic sensitivity 10–15% Faecal chymotrypsin seems to be a reliable screening test for exocrine pancreatic function Border values or low values indicate a direct evaluation of exocrine pancreatic function The simple test meal (water) method with determination of trypsin in duodenal juice gives from a practical point of view good information of the exocrine pancreatic function

The following plan of investigation is proposed Step 1 careful clinical history and examination Step 2 analysis of faeces for Giardia lamblia entero-pathogenic microorganisms and chymotrypsin sweat test Step 3 peroral small intestinal biopsy and/or duodenal juice analysis and finally—if steps 2 and 3 give normal results Step 4 re-evaluation of dietary history and tests to detect any food intolerance (e g carbohydrate)

KEY WORDS Malabsorption coeliac disease small intestinal biopsy exocrine pancreatic function duodenal juice trypsin amylase xylose tolerance test lactose tolerance test B-folate faecal fat faecal chymotrypsin

During recent decades various tests have been developed and recommended for use as diagnostic means to investigate malabsorption Peroral small intestinal biopsy is one of the most important methods in discovering *enterogenic* malabsorption In recent years it has become a routine method in most children s hospitals It is generally agreed that peroral small intestinal biopsy is the only reliable method for the diagnosis of coeliac disease Only the examination of aspirates of duodenal juice can establish *pancreatic* malabsorption

None the less several more or less reliable and often time consuming tests—single or combined—have been proposed as screening tests to select children for further investigation and to avoid unnecessary biopsy and/or duodenal intubation (2 3 10 21 29 33 36 37 44) However there are different opinions about this (15 37 40 41 42)

In order to find the simplest and most reliable method for a diagnosis we started a prospective investigation in 1968 The children admitted to the hospital suspected of malab-

health service and of educational programs By combining a high degree of imagination with enormous working capacity Bo Vahlquist had always been able to make impressive achievements in all fields in which he has engaged himself The results of his research work on infant nutrition were followed by important practical applications leading to an improvement in the health situation of innumerable children all over the world

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Stig Sjolin

ble 2 Diagnostic specificity and sensitivity xylose tolerance test lactose tolerance test folate S iron S albumin and faecal fat in ildren with suspected malabsorption (the ts and biopsy performed at admission)[a]

	Diagnostic specificity (per cent true positive test) in various degrees of abnormal mucosa		
	(%)	(%)	(%)
thological			
ylose TT	50	64	9[illegible]
actose TT	45	60	91
folate	75	84	97
iron	40	48	85
-albumin	[illegible]	73	9[illegible]
'aecal fat	[illegible]	89	100
iopsy			
ievere damage (S)	S	S	S
Moderate damage (M)	M	M	M
Slight damage (SL)	SL	SL	SL
Normal (N)	N	N	N
Normal			
Xylose TT	82	69	26
Lactose TT	85	75	25
B folate	81	73	22
S-iron	71	59	20
S albumin	[illegible]	57	15
Faecal fat	78	57	17
	(%)	(%)	(%)
	Diagnostic sensitivity (per cent true negative test) in normal and in various degrees of abnormal mucosa		

Example If xylose tolerance test is abnormal the probability of a severely damaged mucosa is 50% and of a slightly moderately *or* severely damaged mucosa 9?% On the other hand if the test is normal the probability of a normal mucosa is 26%

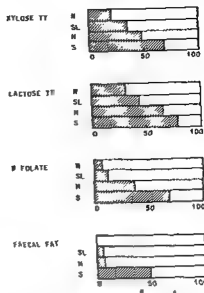


Fig 1 Results of laboratory tests (▨ pathological per cent) related to mucosal morphology (N=normal SL=slight M=moderate S=severe damage)

more specimens taken a few centimetres apart were studied histologically 92 were of similar appearance In 2 the morphology differed one step (e g from normal to slight damage from slight to moderate damage) A heterogeneity of the same degree was seen in 2 cases in the same biopsy specimen In none was there a variation from normal to severely damaged mucosa Thus heterogeneous morphology was found in 4 children two of them being 6 weeks old

The results of the determination of faecal fat (patients with pancreatic affection excluded) xylose tolerance test lactose tolerance test B folate S iron and S albumin were related to morphology of the small intestinal mucosa at admission Table 2 gives the probability of an abnormal finding in an intestinal biopsy when one of these tests is pathological (diagnostic specificity) and also the probability of a normal histology when the test is normal (diagnostic sensitivity) For instance if a child has an abnormal xylose tolerance test the probability of a severely damaged mucosa is 50% on the other hand if the xylose tolerance test is normal the probability of a normal mucosa is 26% As the table shows the only diagnostic test specific up to 100% is faecal fat determination however its diagnostic sensitivity is low (17%)

Combinations of these tests increase the figures When xylose tolerance test lactose tolerance test B folate and faecal fat are combined the diagnostic specificity is 100% and the diagnostic sensitivity 41 88 and 94% in the various morphologic groups (compare Table 2)

Table 1 *Morphology of small intestinal biopsy specimens (flexura duodeno jejunalis) at admission and at follow up*

	No of biopsies		
	at admission	at follow up	total
Normal mucosa	32	21	53
Slightly damaged mucosa	66	27	93
Moderately damaged mucosa	21	22	43
Severely damaged mucosa	49	9	58
Not evaluable	4	2	6
Total	172	81	253

sorption were studied with various tests Small intestinal biopsy and duodenal intubation for collecting duodenal juice were made because of clinical symptoms and signs We present our experiences from this study

MATERIAL

180 children (98 boys and 82 girls) living in Malmo (~250 000 inhabitants) aged 1 month to 15 years (mean 20 months) were admitted to the Paediatric Department in Malmö in 1968 to 1976 suspected of malabsorption The suspicion was raised because of failure to thrive abnormal stools during more than 3 weeks vomiting and/or abdominal distension

METHODS

Laboratory tests

The tests included S electrophoresis S alkaline phosphatase S-calcium S phosphate S iron total iron binding capacity B folate (normal value B folate ≥70 mmol/l) daily faecal fat excretion on normal diet on 3 day specimens (20) (normal value ≤ 4 g/day) D xylose tolerance test (dose 15 g/m² body surface) with determination of blood xylose after 30 and 60 minutes (30 35) (normal values 30 min ≥1 0 60 min≥1 7 mmol/l) lactose tolerance test (dose 2 g/kg body weight in 10% solution) (normal value B glucose increase ≥1 1 mmol/l within 60 min) faecal chymotrypsin (18) (normal value ≥0 32 mkat/kg border value 0 22–0 32 mkat/kg) and sweat test (pilocarpin iontophoresis) (normal value sodium ≤60 mmol/l) The stools were cultured for entero-pathogenic microorganisms and examined for Giardia lamblia and Entamoeba histolytica

Small intestinal biopsy

The children fasted for at least 6 hours They were premedicated with alimemazine 2 mg/kg body weight orally and/or with phenobarbital 30 mg <1 year of age and 60 mg>1 year of age rectally Children older than 4 months were intubated through the nose and local anaesthesia was obtained with lidocaine The biopsy was taken by a hydraulic capsule (31) at the duodeno-jejunal flexure under fluoroscopic control On average three specimens were taken from this region on each occasion The biopsy specimen was oriented on a millipore filter (25) and fixed in 4% formol solution with short after fixation in Bouin's solution After examination and photography in a dissecting microscope the biopsy was serially cut into 5-6 μm sections Alternative slides were stained with haematoxylin and erythrosin with van Gieson stain and with periodic acid Schiff according to McManus The best oriented central cores of the specimens were used for assessment The biopsies were reviewed without knowledge of clinical findings and of the results of laboratory tests They were classified according to Alexander (1) *Normal mucosa* villous (finger and leaves) (Grade I) *slightly damaged mucosa* ridged (Grade II) *moderately damaged mucosa* convoluted (Grade III) *severely damaged mucosa* flat mosaic (Grade IV)

Duodenal juice

The infants fasted for 4 hours and the children overnight The duodenal intubation was usually performed directly after taking the small intestinal biopsy The duodenal tube (Salem sump tube—Sheridan) was introduced with the aid of a Seldinger guide wire lubricated with silicone grease and the tip was in position in the transversal or ascending part of the duodenum (fluoroscopic control) within a few minutes The duodenal juice was collected by siphon in crushed ice before and up to 60 minutes after a test meal of water (50–250 ml depending on age) (5 26) The juice was analysed either directly or after storage at −70°C for pH for trypsin activity (substrate N benzoyl DL arginine p nitroanilide HCl BAPNA) (Sigma) (13) and for amylase activity (Phadebas Amylase test Pharmacia Sweden) (11)

RESULTS

Peroral small intestinal biopsy was done in 177 children on 253 occasions about 750 specimens being taken No complications such as haemorrhage and perforation occurred In 8 children the clinical history indicated pancreatic insufficiency therefore no biopsy was taken but duodenal juice was collected Table 1 presents the morphology of the biopsy specimens at admission and at follow up (The follow up biopsies were taken mostly to note the effect of elimination and the reintroduction of gluten in the diet) Six biopsies (4 at admission and 2 at follow up) could not be examined adequately because of mechanical laceration However a moderately or severely damaged mucosa could be excluded in the 4 biopsies taken at admission On 94 occasions two or

Table 4 *Trypsin and amylase activities in duodenal juice from children with normal border and abnormal values of faecal chymotrypsin*

	No of children		
	Normal value	Border value	Abnormal value
Faecal chymotrypsin	132	2	8
Duodenal juice analysed in	44	2	8
Normal trypsin activity	44	1	3
Normal amylase activity	44	2	4

chymotrypsin was normal in 132 of 142 children 2 had border values and 8 abnormally low values (Table 4) Duodenal juice was analysed in 44 of 132 children with normal faecal chymotrypsin In all 44 the trypsin and amylase activities were normal (Table 4) One boy aged 6 months (cystic fibrosis) with border value of faecal chymotrypsin had low trypsin activity (47 µg/ml) normal amylase activity and clinical signs of pancreatic insufficiency The other child with a border value of faecal chymotrypsin but normal trypsin and amylase activity developed pancreatic insufficiency two years later One boy aged 16 months with low faecal chymotrypsin had a remarkably low amylase activity (see Fig 2) but normal trypsin activity (~800 µg/ml)

In three children the symptoms were caused by infestation with *Giardia lamblia* and in one infant by *Entamoeba histolytica*

DISCUSSION

In this study the laboratory tests are related to small intestinal mucosal structure The first question is how relevant are the biopsy findings? Although the photographic documentation of the dissecting microscopic appearance of the intestinal mucosa facilitates the classification of the findings the combinations of macroscopic and histological findings are always open to subjective appraisal At the review of the biopsies a good agreement was found between the primary histological diagnosis and the recoding especially between the grades normal mucosa and severe mucosal damage Thus it was not thought necessary to engage two pathologists in the re classification Patchiness (heterogeneity) of mucosal lesions was seen in 4 patients only (two being very young and without coeliac disease) thus in less than 2% of the specimens Varying mucosal damage in the upper part of the intestine seems to be of less importance for the results However we do not know the extension of the mucosal damage

Especially for one group of patients namely adults with dermatitis herpetiformis multiple biopsies of the full length of the small intestine has shown patchy lesions and also great variation in extent of the mucosal damage (9) In that study laboratory evidence of malabsorption was absent in many patients this was best explained by limited length of small intestinal involvement To the best of our knowledge studies of this type have not been performed in the primary stage of malabsorption in children

Our prospective study shows that the laboratory tests for detecting enterogenic malabsorption have a low diagnostic sensitivity Thus a normal result of each of these tests says very little about the condition of the intestinal mucosa Combinations of these tests increase the sensitivity by 10 to 15% but it is not enough to justify their use An increased excretion of faecal fat (with a normal faecal chymotrypsin) and/or low B folate almost always means an abnormal mucosa (=high diagnostic specificity) findings which agree with other studies (29 39 44) In contrast to the results of McNeish & Willoughby (29) we found that a normal B folate by no means excludes the possibility of a severely damaged mucosa

The diagnostic specificity of xylose and lactose tolerance tests is lower than that of faecal fat and B folate The number of false positive results in lactose tolerance test was of the same order as found by Krasilnikoff et al (22) They concluded that the flat blood glucose

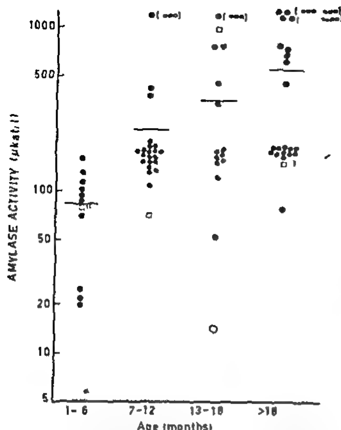


Fig 2 Amylase activity (maximum values 1 μkat=60 IU) in duodenal juice in various age groups □ Children with low trypsin activities □[1] salivary amylase 15% □[2] salivary amylase 0% O boy with normal trypsin but low faecal chymotrypsin salivary amylase 15% —=mean value (children with pancreatic insufficiency excluded)

At the follow up biopsies xylose tolerance test was done on 31 biopsy occasions The mucosa was severely damaged on 7 and moderately damaged on 12 The test was abnormal on only one occasion (moderately damaged mucosa)

Fig 1 illustrates the result if the morphological picture is the starting point and the findings of the laboratory tests are given in the different morphologic groups that is a retrospective approach to the problem and is most commonly used in previous studies The xylose tolerance test and lactose tolerance test[1] were false positive in 19 and 30%

One third of the children with severely damaged mucosa and one fifth with normal mucosa had low S iron values S albumin was decreased in 31% of the children with severely damaged mucosa

Table 3 *Trypsin content (maximum values) in duodenal juice in children with suspected malabsorption (pancreatic insufficiency excluded) and in children with pancreatic insufficiency*

	Age (months)	Trypsin content (μg/ml) *n*	Mean±S D	Range
Suspected malabsorption	1–6	12	739±335	130–1 [illegible]00
	7–12	20	873±397	[illegible]35–1 900
	>12	36	663±376	17[illegible]–1 600
Pancreatic insufficiency		[illegible]	70 60 47 0 0 0	

Alkaline phosphatase was transiently increased in 8 children with moderately or severely damaged mucosa In only one patient did clinical and radiological findings reveal rickets

Duodenal juice was collected from 76 children and analysed for trypsin and amylase activity Trypsin activity was decreased (<100 μg/ml) in 6 (5 with cystic fibrosis one with Shwachman syndrome) (Table 3) As Fig 2 shows amylase content varied considerably In 3 children with cystic fibrosis the amylase activity was low or not measurable whereas the other 3 with low trypsin activity had an amylase content comparable to that of the children without pancreatic affection[2] Faecal

[1] Intestinal disacchandase activities were analysed in 64 children (A Dahlqvist Institute of Nutrition University of Lund) One boy showed an isolated lactase deficiency his lactose tolerance test is excluded The other 108 had no clinical history which indicated isolated *lactase deficiency*

[2] Isoenzymes of amylase (salivary and pancreatic amylase) were analysed in duodenal aspirates from 12 randomly chosen children In 3 no salivary amylase could be detected in 3 salivary amylase contributed with 5–15% in 2 20–30% in 3 40–50% and in one 95% of total amylase activity In 2 children with pancreatic insufficiency the salivary amylase activity was zero and 15% of the total finally in one boy with low total amylase activity 15% was of salivary origin (see Fig 2) (Dr G Skude Department of Clinical Chemistry Kalmar Hospital Kalmar Sweden)

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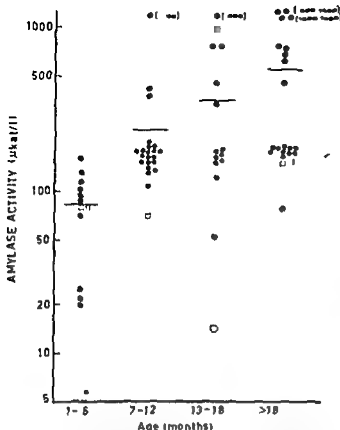


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Pancreatic insufficiency		6	70 60 47	
			0 0 0	

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o balloons ■ the method most commonly ed in children (6 15 23 34 37) despite it ing more complicated to perform than the eal test according to Lundh (26) The pan eozymin-secretin test gives more accurate formation of pancreatic exocrine function determination of volume and bicarbonate so can be done (14) However comparison f the two methods performed on adults has hown the meal test as a more potent stimulus o pancreatic secretion of enzymes (45) com arable results after augmented pancreozymin est or a test meal but most reliable result fter augmented secretin dose (17) and no dif erence between the mean concentrations of rypsin amylase and lipase (27) As the test neal method is simple and safe and entails little discomfort we therefore preferred it Water has been found to give the same tryp sin content in duodenal juice as after a meal with fat carbohydrate and protein (5 24 28) and as it simplified the method still more we used water instead of formula

Similar ■ Ingomar & Terslev (19) we found a great variation of amylase activity With the method used salivary amylase can contribute to the activity in adults (40) and as found in this study also in children therefore determi nation of amylase without assessment of iso-enzymes is of doubtful value when using the test meal method Some pancreatic enzymes such as lipase and amylase develop during the first years of life whereas trypsin activity is well-developed at least at the age of one month (6 14 45) The great variation in for instance amylase activity and the varying re sults of the enzyme analyses in the children with border or low values of faecal chymo-trypsin might indicate great inter individual and mutual variation sin the maturation of the enzymes Such an uneven enzyme profile could be the cause of diarrhoea in some chil dren especially at the age between 6 and 18 months when the diet is increasingly similar to that of adults Malabsorption can also be caused by isolated deficiency of one pancrea tic enzyme (e g amylase lipase trypsinogen)

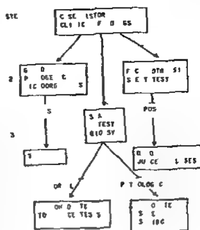


Fig 3 Plan of investigation of suspected malabsorption in children

or enterokinase (15) However to assess the various pancreatic enzyme activities (amylase carboxypeptidase chymotrypsin elastase lipase colipase and trypsin) would be too laborious to be used in routine work The vari ous enzymes can be detected in electrophore sis of duodenal juice and a simple and rapid method has been developed (8) We think that electrophoresis of the duodenal juice com bined with determination of trypsin activity gives in a simple way good and broad in formation of the exocrine pancreatic function

CONCLUSION

This study has shown that when on clinical bases we suspect enterogenic malabsorption in a child we cannot rely on a normal result of xylose tolerance test lactose tolerance test B folate S albumin S iron and faecal fat An abnormal result especially of B folate or faecal fat indicates with great probability an abnormal mucosa In both situations we must perform an intestinal biopsy Therefore we propose the plan of investigation given in Fig 3 We wish to stress the importance of a care ■ clinical history with detailed analyses of symptoms (including weight and length curves) related to diet (Step 1) Step 2 in cludes analyses of faeces for entero-patho-

Table 5 *Final diagnosis in children with suspected malabsorption*

	No of children
Coeliac disease	32
Coeliac disease+cystic fibrosis	1
Suspected coeliac disease	12
Sibling to children with coeliac disease+diarrhoea	2
Cow s milk allergy (protein intolerance)	35
Food allergy (intolerance to cow s milk protein soy protein fish egg fruits etc)	20
Cystic fibrosis	5
Shwachman s syndrome	1
Acute pancreatitis	1
Malabsorption UNS	35
Post infectious diarrhoea[b]	11
Yersinia enterocolitica and *E coli* infection	4
Giardia lamblia	3
Entamoeba histolytica	1
Saccharase–isomaltase deficiency	2
Congen severe familial lactose intolerance (later lactase deficiency)	1
Disaccharide intolerance (uria) with vomiting	2
Mb Hirschsprung	1
Failure to thrive+eczema	2
+ectodermal dysplasia	1
+myelomeningocele	1
+preterm SGA	1
+sideropenia	2
Short stature+diarrhoea[a]	2
+VOC	1
Diabetes insipidus	1
Total	180

[a] Children usually 7–18 months with diarrhoea mood changes temporary weight standstill Spontaneous recovery at age 18–24 months

[b] Prolonged diarrhoea after an acute debut as gastroenteritis Return to normal diet gives diarrhoea up to 2–3 months after the debut

curve after peroral load was caused by a slow gastric emptying rate In our study 15 of 16 children with normal or slightly damaged mucosa had a normal lactase activity the flat blood glucose curve being due to an isolated lactase deficiency in one child only The false positive xylose tolerance tests might in the same way be explained by a slow gastric emptying rate Our experience with xylose tolerance test is similar to that of Sladen & Kumar (41), Schmerling (38) and Harms (16) but disagrees with that of Rolles et al (36) who gave 5 g xylose to all children less than 30 kg body weight and found that a blood level of less than 20 mg/100 ml (=1 35 mmol/l) after one hour very well separated the children with flat mucosa (coeliac disease) from those with normal mucosa In fact only one child (with coeliac disease) had a value on the "wrong" side of the blood level Our xylose dose (15 g/m²) is somewhat higher (about 7 5 g to a one year old child) Decreasing our normal level after one hour to 1 35 mmol/l did not change the diagnostic specificity but gave a lower diagnostic sensitivity of the test

One important reason for these observed variations may be different composition of the clinical materials Our children were admitted from children s welfare clinics or out patient clinics without any intervenient delay Thus they were studied in an early phase of the disease where the damage of the small intestinal mucosa could be of limited extent Slight forms of for instance coeliac disease are diagnosed the mean age at diagnosis of coeliac disease was 15 months with 70% of the children less than 12 months old at diagnosis Anderson & Burke (3) reported the same mean age in their coeliac children but with 50% less than 1 year old at diagnosis The frequency of diagnosed coeliac disease in Malmo became about three times higher after the introduction of peroral small intestinal biopsy in the hospital in 1968 (Berg & Lindberg to be published) The composition of the material is comparable to that of regional children s hospitals but probably not with those of specialized clinics in this part of the world Table 5 lists the children s final diagnosis

Determination of faecal chymotrypsin seems a reliable screening method for pancreatic malabsorption 44 children with normal chymotrypsin had normal activities of trypsin and amylase in duodenal juice a result in agreement with earlier reports (4 7 12 32) Border values or low faecal chymotrypsin is an indication for a direct evaluation of pancreatic exocrine function The pancreozymin–secretin test with two tubes (one in stomach and one in duodenum) or with one triple lumen tube and

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genic microorganisms Giardia lamblia chymotrypsin and a sweat test Abnormal sweat test and/or abnormal faecal chymotrypsin indicates direct evaluation of exocrine pancreatic function If the Step 2 results are negative or if the clinical history and examination indicates enterogenic malabsorption peroral small intestinal biopsy is directly performed With appropriate premedication and with some experience it is done easily and quickly (rarely more than 30 minutes) and it is safe when capsules of paediatric size (with small hole ≤Ø2 mm) are used With the aid of a magnifying glass or a dissecting microscope a normal or a flat mucosa can immediately be diagnosed These procedures can be performed as out patient procedures thus reducing expenses Finally and of more importance our experience is that the procedure of peroral intestinal biopsy or of duodenal intubation is of less discomfort for the child than that of a hospital stay and of the various laboratory tests

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HAEMATOPOIETIC STEM CELLS (CFUc) IN HUMAN CORD BLOOD

G PRINDULL B PRINDULL and N v d MEULEN

From the Department of Paediatrics University of Gottingen Gottingen West Germany

ABSTRACT Prindull G Prindull B and Meulen v d N (Department of Paediatrics University of Gottingen Gottingen West Germany) Haematopoietic stem cells (CFUc) in human cord blood Acta Paediatr Scand 67 413 1978.—Colony forming units (CFUc) giving rise to myelocytic colonies in methylcellulose cultures were found among non adherent mononuclear cells of human cord blood with a frequency of one in 1678 The number decreased markedly during the first 8 to 10 days of life They were rarely detected in adult blood by this technique

KEY WORDS Haematopoietic stem cells CFUc cord blood

The existence of haematopoietic stem cells has been substantiated most convincingly by the successful transplantation of bone marrow in patients with aplastic anaemia and leukaemia (3 5 21) Since in the foetus the haematopoietic system is in a state of physiologic proliferation cord blood might constitute another source of haematopoietic stem cells In this study we have demonstrated colony forming myelopoietic stem cells (CFUc) among cord blood lymphoid cells

MATERIALS AND METHODS

Subjects

Three groups of individuals were studied Group I Twelve normal full term newborn infants Group II Twelve infants 8–10 days old who had been hospitalized for diseases not involving the immunohaematopoietic system and who were clinically well at the time of the test Group III Twelve healthy adult volunteers from our medical staff

Methylcellulose cultures (11 12)

Fifteen ml of blood from each individual was processed Cord blood and venous blood from infants of Group II was collected with an equal amount of a 30 g/l dextran solution (molecular weight 250000 C Roth Karlsruhe West Germany) in 8 5 g/l saline Blood from adults was collected with heparin The blood was processed not later than one hour after collection until which time it was kept at 4°C

The blood was allowed to sediment in plastic syringes for 1 hour at 37°C The serum and buffy coat were collected and centrifuged at 330 g for 15 min The cells were resuspended and washed in alpha medium (alpha MEM (Eagle modified with Earle s salts Flow Laboratories Irvine Scotland) with 250 g/l fetal calf serum (Difco Laboratories Detroit USA) The cell suspension was partially cleared of non lymphoid cells by allowing the cells to adhere to plastic Petri dishes at 37 C in an atmosphere containing 75 ml CO_2/l of air for 3 hours (14)

From each individual four plates (disposable plastic dishes 35 mm in diameter Greiner & Sons Nurtingen West Germany) were prepared Each plate contained 0 4 ml of a 20 g/l methylcellulose (Methocel MC premium 4000 CPS Dow Clinical Co Midland Mich USA) gel in distilled water 0 05 ml of a 200 g/l bovine serum albumin solution (Behringwerke Marburg West Germany deionized) 0 25 ml of alpha medium containing 100000 non adherent mononuclear cells and 0 3 ml of foetal calf serum Conditioned medium was not added The plates were incubated at 37°C in an humidified atmosphere with 75 ml CO_2/l of air for 10 days

During their incubation period cultures were observed regularly at 24 hour intervals with an inverted microscope After 10 days of incubation final counts of all colonies in each plate were made In addition smears were prepared from the colonies and were stained with Wright s and peroxidase stains

RESULTS

During the first days of incubation single cells were evenly distributed throughout the methylcellulose medium There were no cell clumps clusters or aggregations This was taken as evidence that colonies developing subsequently were derived from single cells of the original cell suspension namely from CFUc Colony formation was evident from the

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Fig 1 (*a*) Small colony from a methylcellulose culture (*b*) Myelocytes and metamyelocytes (*c*) segmented and band neutrophils (*d*) dividing myelocytic cell from smears prepared from the colonies

7th day of culture (Fig 1*a*) and by the 10th day colonies of 50 and more cells had formed Cytologic examination revealed peroxidase positive myelocytic cells (Fig 1*b*) capable of full maturation (Fig 1*c*) In addition blast cells and cells in mitosis (Fig 1*d*) also were present

The results are summarized in Table 1 The mean number of colonies from cord blood was 59 6±14 9 per plate Since each culture had been plated from 100000 non adherent mono nuclear cells, it follows that there were about one CFUc among 1678 cord blood non ad herent mononuclear cells In infants 8 to 10 days old, the number of circulating CFUc de creased markedly approaching adult levels where we have rarely found them

DISCUSSION

Increased haematopoietic proliferation of the bone marrow is reflected in the peripheral blood by an elevated number of circulating stem cells For example in phenylhydrazine induced haemolytic anaemia when the bone marrow is strongly stimulated increased num bers of haematopoietic stem cells (9) and of cells in spontaneous DNA synthesis (8) are found in the circulating blood In patients re lapsing from acute lymphocytic leukaemia the number of circulating CFUc is elevated (2 19) indicating that in this situation there is in creased proliferation in the bone marrow not only of neoplastic cells but also of normal haematopoietic cells Similarly in cancer pa

Table 1 *Number of CFUc in cultures from cord blood from infants 8 to 10 days old and from adults*

Cord blood no	Colonies per plate	Infants no	Colonies per plate	Adults no	Colonies per plate
1	54	1	74	1	0
2	59	2	60	2	0
3	68	3	0	3	7
4	[illegible]	4	4	4	0
5	35	5	0	5	3
6	76	6	0	6	0
7	54	7	0	7	0
8	55	8	22	8	0
9	49	9	45	9	0
10	70	10	20	10	0
11	55	11	0	11	8
12	97	12	4	12	0
Mean (±δ)	59 6 (±14 9)				

tients after termination of successful chemotherapy haematopoiesis in the bone marrow is stepped up with a simultaneous rise in circulating CFUc (20)

During foetal development the bone marrow is in a state of physiologic proliferation securing oxygen supply to the rapidly growing tissues and building up the myelocytic (23) and lymphatic system (16) From animal experiments we know that foetal blood contains more than 100 times as many stem cells affording irradiation protection than are present in adult blood (1) The present experiments show that in human cord blood the number of CFUc is larger than in adult blood by approximately the same factor as in laboratory animals (13 17) There was one CFUc among 1678 non adherent mononuclear cells from cord blood Within a few days following birth CFUc of peripheral blood decrease in number (Group II) This decrease is not uniform in all infants There is a pronounced individual variation for which we have at present no explanation By the end of the first month of life CFUc reach the low levels found in the adult (7 22)

The CFUc of cord blood detected by the present technique were myelopoietic progenitor cells Colony stimulating factor (CSF) necessary for myeloid colony formation must have been furnished by contaminating monocytes (22) although we have found no correlation between the number of monocytes in the original cell suspension and the number of subsequently developing colonies The addition of CSF did not increase the number of colonies formed from cord blood (unpublished observations) This has also been shown for adult CFUc by Tebbi and co workers (22) No erythropoietic colonies were seen

The presence of CFUc in human cord blood raises the problem of their morphological identity In monkeys Moore and co-workers (15) have identified the in vitro colony forming cell as a transitional lymphocyte with a cell diameter of 9–11 μm basophilic cytoplasm and a round central leptochromatic nucleus During recent years work from Yoffey (6 24) from others (4 10) and from our own laboratory (17 18) has provided firm evidence pointing to the transitional cell (24) as the most likely candidate for the activated haematopoietic stem cell in man

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3	68	3	0	3	7
4	48	4	4	4	0
5	35	5	0	5	3
6	76	6	[illegible]	6	0
7	54	7	0	7	0
8	55	8	22	8	0
9	49	9	45	9	0
10	70	10	70	10	0
11	55	11	0	11	8
12	92	12	4	12	0
Mean (±δ)	59 6 (±14 9)				

tients after termination of successful chemotherapy haematopoiesis in the bone marrow is stepped up with a simultaneous rise in circulating CFUc (20)

During foetal development the bone marrow *is in a state of physiologic proliferation* securing oxygen supply to the rapidly growing tissues and building up the myelocytic (23) and lymphatic system (16) From animal experiments we know that foetal blood contains more than 100 times as many stem cells affording irradiation protection than are present in adult blood (1) The present experiments show that in human cord blood the number of CFUc is larger than in adult blood *by approximately* the same factor as in laboratory animals (13 17) There was one CFUc among 1678 non adherent mononuclear *cells from* cord blood Within a few days *following birth* CFUc of peripheral blood decrease in number (Group II) This decrease is not uniform in all infants There is a pronounced individual variation for which we have at present no explanation By the end of the first month of life CFUc reach the low levels found in the adult (7 22)

The CFUc of cord blood detected by the present technique were myelopoietic progenitor cells Colony stimulating factor (CSF) necessary for myeloid colony formation must have been furnished by contaminating monocytes (22) although we have found no correlation between the number of monocytes in the original cell suspension and the number of subsequently developing colonies The addition of CSF did not increase the number of colonies formed from cord blood (unpublished observations) This has also been shown for adult CFUc by Tebbi and co workers (22) No erythropoietic colonies were seen

The presence of CFUc in human cord blood raises the problem of their morphological identity In monkeys Moore and *co-workers* (15) have identified the in vitro colony forming cell as a transitional lymphocyte with a cell diameter of 9–11 μm basophilic cytoplasm and a round central leptochromatic nucleus During recent years work from Yoffey (6 24) from others (4 10) and from our own laboratory (17 18) has provided firm evidence pointing to the transitional cell (24) as the most likely candidate for the activated haematopoietic stem cell in man

ACKNOWLEDGEMENT

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SERUM PROLACTIN FSH AND LH DURING PUBERTY IN GIRLS AND BOYS

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ABSTRACT Apter D Pakarinen A and Vihko R (Department of Medical Chemistry University of Helsinki SF-00170 Helsinki 17 the Department of Clinical Chemistry University of Oulu SF 90220 Oulu 2? Finland) Serum prolactin FSH and LH during puberty in girls and boys Acta Paediatr Scand 67 417 1978 —Serum prolactin follicle stimulating hormone and luteinizing hormone were determined in 200 girls and 80 boys The boys have been examined on three occasions at one year intervals and the girls twice at 1 5 year intervals In girls serum FSH rapidly increased in the youngest age groups (7 5–11 5 years) whereas in boys the increase took place later and the first significant increase was seen between age groups 9 5 and 12 5 years In girls a rise in serum LH took place later than that of FSH (between 10 5 and 11 5 years) and LH peaked at 13 0–13 5 years In boys the timing in the changes of serum LH closely resembled that of FSH The girls displayed a significant increase in serum prolactin between 7 5 and 8 5 years and this was followed by a slow progressive increase In the group of boys serum prolactin did not show any significant changes In girls there was a correlation between serum LH and body weight as well as calculated fat amount and body fat percentage early in puberty There was no correlation between serum LH and chronological or bone age in this age group which suggests that the correlation found is not due to age related parallel phenomena

KEY WORDS Gonadotropins FSH LH prolactin puberty

The correlation between pubertal and hormonal changes has recently attracted great attention One important aspect includes studies on the pituitary secretion of gonadotropic hormones (8 17 25) and more recently prolactin (3 19 20 26) There is now a rather uniform view on the serum levels of gonadotropins during puberty whereas in the case of prolactin conflicting results have been obtained It has been demonstrated that serum prolactin is higher in grown up women than in prepubertal girls (21) and some studies have confirmed a gradual increase in its serum level (3) whereas others have not observed any such differences (9 20) In the case of males the consensus of opinion seems to be that no major changes take place in the prolactin level during pubertal development (7 19)

In elucidating the hormonal changes and concomitant physical and psychic phenomena during puberty longitudinal studies with sufficiently large numbers of subjects can be expected to provide the most relevant information on their relationships As a part of such a study we report here the serum prolactin follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in groups of 200 girls and 80 boys The boys have been examined on three occasions at one year intervals and the girls twice at 1 5 year intervals Data on serum steroid measurement in girls have already been published (1)

MATERIALS AND METHODS

Subjects

Details of the female pubertal population have been described previously (1) Of the initial 700 girls 7–17 years

Table 1 *Serum FSH LH and prolactin in girls according to bone age*

Bone age (years)	7 5	8 5	9 5	10 5	11 5	12 5	13 5	14 5	15 5	16 5	17 5	18 5
Number of subjects	14	19	16	35	25	32	41	20	33	38	76	7
FSH IU/l												
Mean	2 0	2 2	3 5	4 5	8 0	8 0	9 0	9 3	8 7	8 8	8 6	8.5
S E M	0 16	0 23	0 60	0 46	0 76	0 44	0 39	0 80	0 45	0 41	0 41	0 79
LH IU/l												
Mean	5 9	5 5	6 0	6 3	8 3	9 6	13 2	11 2	11 9	17 6	13 4	13.3
S E M	0 81	0 46	0 45	0 34	0 64	0 60	0 71	0 62	0 95	0 57	0 74	1.35
Prolactin												
Mean	6 2	8 5	7 8	8 0	7 2	8 2	9 5	9 3	8 5	10 5	10 3	9 1
S E M	1 10	0 98	1 14	0 73	0 74	0 69	0 94	0 78	0 71	1 11	0 97	1 71

In postmenarcheal girls the values are from samples taken between days 6–9 of the menstrual cycle * ** and *** indicate significances of differences $p<0\ 05$ $p<0\ 01$ and $p<0\ 001$ respectively

old 140 took part in a second examination 1 5 years after the first The grading of pubertal stages according to Tanner (23) bone age determination (15) and health controls for girls were as previously described (1) The bone age group 18 5 years consists of girls from the second sample collection whose bone age in the first investigation had already reached 17 years This was done because the exact determination of bone age after 17 years is not possible with the method used In postmenarcheal girls two samples were drawn on days 6–9 and 20–23 of the same menstrual cycle In this study only samples drawn at the beginning of the cycle will be reported

The 80 boys participating in this study were 8–18 years of age during the first part of the study Of these 67 were examined twice and 44 three times at one year intervals The boys were healthy pupils attending two schools in Oulu and took part in the study on a voluntary basis The pubic hair and genital development stages were graded according to Tanner (23) Testicular volume was measured according to From Hansen & With (13)

Permission for the examinations and for drawing blood samples was obtained in written form from the parents of the girls and boys examined and the school and health authorities of the cities of Helsinki and Oulu

Hormone determinations

Serum FSH LH and prolactin were estimated using CEA IRE SORIN (CIS) kits (Département des Radioéléments B P no 21 91190 Gif sur Yvette France) and the analyses were performed following the manufacturer s instructions without modification In each analysis batch samples from all age groups were incorporated and the quality of the series was monitored by the inclusion of low medium and high controls in each batch Other details have been published recently (16)

The statistical treatment of the results was performed at the Department of Data Processing University of Oulu Groups were compared using two tailed Student s *t* test and Wilcoxon test for pair differences

RESULTS

Fig 1 shows the changes in serum FSH L and prolactin in girls expressed in a mix longitudinal approach (modified from Tann & Gupta, 24) Thus, the ratios of individu changes in hormone concentration and bo age are calculated and the mean annu changes are then consecutively added to t mean value of the previous age group beg ning from the youngest group Table 1 giv the actual mean concentrations in annual bo age groups for FSH LH and prolactin in

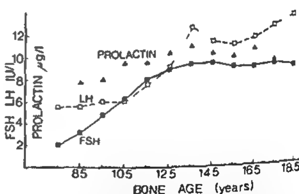


Fig 1 Serum FSH (IU/l) LH (IU/l) and prolactin (µ in pubertal girls The changes have been expressed i mixed longitudinal manner (see ref 24) in which ratios of changes in hormone concentrations and bone in each individual are first calculated The mean ann changes are then added to the previous mean beginn from the mean actual concentration of the youngest group

Table 2 *Serum FSH LH and prolactin in boys according to chronological age*

Age (years)	8 5	9 5	10 5	11 5	12 5	13 5	14 5	15 5	16 5	17 5	18 0–19 9
Number of subjects	7	14	[illegible]	2[illegible]	19	23	17	19	18	18	14
FSH IU/l											
Mean	0 7	0 9	1 0	1 9	2 6	3 3	4 0	3 4	4 7	5 3	4 4
S E M	0 16	0 12	0 17	0 29	0 36	0 41	0 48	0 36	0 44	0 73	0 91
LH IU/l											
Mean	4 0	3 4	4 [illegible]	5 4	6 4	6 9	8 7	7 9	7 6	7 7	8 4
S E M	0 45	0 [illegible]	0 43	0 47	0 35	0 51	0 60	0 49	0 46	0 76	0 68
Prolactin μg/l											
Mean	7 1	9 6	8 0	7 3	6 5	5 8	6 0	6 2	7 1	7 8	7 4
S E M	1 39	1 70	1 30	1 [illegible]	0 85	0 59	0 69	0 50	0 38	0 94	1 01

and indicate significances of differenced $p<0\ 05$ $p<0$ [illegible] and $p<0\ 001$ respectively

cross sectional manner with the results from the first and second samples combined A good agreement between the two approaches (Fig 1 and Table 1) is seen

The significance of the changes was tested with a Wilcoxon test for paired differences The four youngest age groups (7 0–7 9 8 0–8 9 9 0–9 9 and 10 0–10 9 years of bone age during the first collection) display a significant ($p<0\ 01$) increase in their serum FSH whereas no change is seen after 11 5 years of bone age The pattern for serum LH is different from that of FSH (Fig 1 Table 1) Significant increases (Wilcoxon test for paired differences $p<0\ 05$) are seen from the bone age groups 10 5 11 5 and 12 5 years in the first collection A distinct peak is seen in the age group 13 5 years after which a decrease ($p<0\ 05$) and a second rise to an adult level takes place

The ratio of serum FSH concentration to that of LH increases from 0 4 in the youngest age group to a maximum level of 1 0 in the 11 5 year-old group and declines thereafter to around 0 8 in the older groups A significant increase in serum prolactin (Fig 1) occurred between the two youngest age groups ($p<0\ 05$) followed by a slow progressive increase until the age group 13 5 years

In boys the concentrations of serum FSH LH and prolactin are shown in Table 2 and Fig 2 FSH gradually increases from the youngest age group on The serum LH concentration increases between the age groups 10 5 years to 14 5 years after which no major changes take place The ratio of serum FSH concentration to that of LH increases from an initial value of 0 2 in the youngest boys to 0 5–0 7 in the three oldest groups (Table 2) Serum prolactin level was unchanged until the age group 14 5 years after which an increase is seen (Fig 2) but it was not statistically significant

Fig 3 demonstrates the hormonal levels re

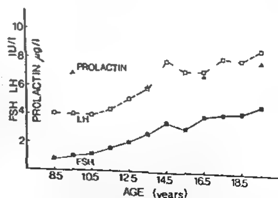


Fig 2 Serum FSH (IU/l) LH (IU/l) and prolactin (μg/l) in pubertal boys The changes have been expressed in a mixed longitudinal way (see ref 24) In this case the mean of the individual annual changes are successively added to the mean actual concentration of the youngest age group

Table 1 *Serum FSH LH and prolactin in girls according to bone age*

Bone age (years)	7 5	8 5	9 5	10 5	11 5	12 5	13 5	14 5	15 5	16 5	17.5	18.5
Number of subjects	[illegible]	19	16	35	25	32	41	20	33	38	76	7
FSH IU/l												
Mean	2 0	2 2	3 5	4 5	8 0	8 0	9 0	9 3	8 7	8 8	8 6	8.5
S E M	0 16	0 23	0 60	0 46	0 76	0 44	0 39	0 80	0 45	0 41	0 41	0 79
LH IU/l												
Mean	5 9	5 5	6 0	6 3	8 3	9 6	13 2	11 2	11 9	12 6	13 4	13 3
S E M	0 81	0 46	0 45	0 34	0 64	0 60	0 71	0 62	0 95	0 57	0 74	1 35
Prolactin												
Mean	6 2	8 5	7 8	8 0	7 2	8 2	9 5	9 3	8 5	10 5	10 3	9 1
S E M	1 10	0 98	1 14	0 73	0 74	0 69	0 94	0 78	0 71	1 11	0 97	1 71

In postmenarcheal girls the values are from samples taken between days 6–9 of the menstrual cycle * * and * indicate significances of differences $p<0\ 05$ $p<0\ 01$ and $p<0\ 001$ respectively

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The statistical treatment of the results was performed at the Department of Data Processing University of Oulu Groups were compared using two tailed Student s *t* test and Wilcoxon test for pair differences

RESULTS

Fig 1 shows the changes in serum FSH LH and prolactin in girls expressed in a mixed longitudinal approach (modified from Tanner & Gupta 24) Thus the ratios of individual changes in hormone concentration and bone age are calculated and the mean annual changes are then consecutively added to the mean value of the previous age group beginning from the youngest group Table 1 gives the actual mean concentrations in annual bone age groups for FSH LH and prolactin in

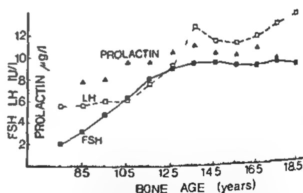


Fig 1 Serum FSH (IU/l) LH (IU/l) and prolactin (µg in pubertal girls The changes have been expressed in mixed longitudinal manner (see ref 24) in which ratios of changes in hormone concentrations and bone a in each individual are first calculated The mean ann changes are then added to the previous mean beginni from the mean actual concentration of the youngest a group

Table 3 *Bone age and serum concentration of FSH and LH according to weight (kg) in girls*

Weight groups	< 5	25–29	30–34	35–39	40–44	45–49	50–54	55–59	≥60
Number of subjects	9	16	[illegible]6	19	[illegible]1	27	3[illegible]	21	22
FSH Mean	[illegible] 0	9	5 1	6 5	9 1	9 0	8 2	8 6	9 1
S E M	0 33	0 38	0 65	0 78	0 60	0 71	0 42	0 74	0 62
LH Mean	4 7	5 8	6 9	8 7	1[illegible] 7	1[illegible] [illegible]	12 4	12 8	11 6
S E M	0 [illegible]0	0 45	0 41	0 73	1 68	1 04	0 80	1 53	0 87
Bone age Mean	8 0	8 8	10 2	11 5	13 5	14 [illegible]	15 8	15 8	16 4
S E M	0 [illegible]6	0 [illegible]6	0 [illegible]4	0 30	0 39	0 42	0 27	0 43	0 23

partly longitudinal manner Out of the initial 200 girls 140 took part in a second examination 1 5 years after the first Correspondingly 67 of the initial 80 boys were examined twice and 44 three times at one year intervals This report presents the results of serum FSH LH and prolactin

The hormonal changes in relation to age followed a different pattern in the two sexes In girls the results obtained confirm the trend observed in earlier studies (8 14 17 25) FSH starts to increase early and continues to increase up to 11 5 years of age by which time an adult concentration level is achieved The LH increase starts later from 10 5 years of age and a peak is seen in the group of 13 5 years of bone age with a second rise to adult levels in the oldest age groups

In the boys the rise in serum FSH starts later than that in girls and the rise in serum LH closely follows that of FSH This simultaneous increase in gonadotropins in boys is different from that in girls and has also been observed in earlier investigations (8 14 19) The first significant increase in the concentrations of these two hormones as compared to younger age groups takes place by 11 5 years of chronological age The increase in serum FSH continues for at least 4 years longer than that in girls the mature level when reached however remains lower

There is also a sex difference during puberty in the FSH/LH ratio the ratio being considerably higher in girls The difference in the timing of the FSH and LH changes is also reflected in the FSH/LH ratio In girls this ratio shows a maximum in the bone age group of 11 5 years (1 0) whereas in boys the ratio shows a gradual increase reaching a maximum of about 0 6–0 7 at 16–18 years of chronological age The sex difference observed in the ratio and its changes also compares well with the established sex difference in LH RH response In girls the FSH response as compared with the LH response is maximal at about 11 years after which the LH response increases In the boys the FSH and LH responses increase in parallel (6)

It is quite well documented that in adults serum prolactin concentration is higher in females than in males (2 21 26) It has also been reported that children have lower prolactin levels than adult females (22) However conflicting data have been reported on serum prolactin levels during puberty In a number of studies no increase in serum prolactin concentration was seen during pubertal development in boys and girls (2 9 19 20) On the contrary Ehara et al (7) found a significant increase in prolactin concentration in girls between 14 and 15 years of age but no changes were seen in boys The prolactin concentration has also been reported to rise steadily with age and pubertal development in girls (3) Possible explanations for the conflicting results include variations in the timing of sam

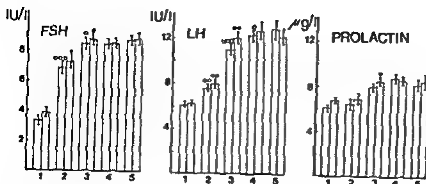


Fig 3 Serum FSH LH and prolactin according to breast (light columns) and pubic hair (dark columns) stages in girls One to three of the symbols O or * indicate $p<0.05$ $p<0.01$ $p<0.001$ significances of difference to the preceding stage for breast and pubic hair development respectively

lated to pubertal stages in girls using breast development and pubic hair growth as the parameters (23) FSH shows the most significant rise between stages 1 and 2 whereas LH does so between stages 2 and 3 Prolactin shows a small increase between stages 2 and 3 ($p<0.05$) Similarly Fig 4 demonstrates the hormonal levels in relation to pubertal stages in boys using genital development and pubic hair growth as the parameters It is seen that FSH increases gradually throughout the pubertal stages whereas LH remains unchanged from genital stage 3 onwards and from pubic hair stage 4 forwards (Fig 4) In contrast to girls serum prolactin demonstrates no change in relation to pubertal stages

Both FSH and LH in girls show a high correlation with chronological age bone age pubic hair and breast developmental stages ($r=0.57$–0.60 for all correlation coefficients $p<0.001$) Prolactin correlates with bone age chronological age and pubertal stage ($p<0.05$)

In prepubertal girls (breast and pubic hair stage 1, bone age <9.5 years) a correlation ($p<0.01$) between LH and body weight as well as calculated fat amount and body fat percentage is seen In this age group LH does not correlate with bone age Longitudinally, when the differences in hormones and physical parameters between the two sample collections are correlated a rise in weight correlates with a rise in LH ($p<0.05$) but not with the other hormones in the whole female population

Table 3 shows the female population grouped on the basis of weight and for a comparison with Table 1 the mean bone age in the corresponding weight groups has also been calculated An increase in serum LH is seen from the first group onwards and the difference between the first and the third group is significant ($p<0.01$)

In boys serum FSH and LH correlates with chronological age pubic hair stages total testicular volume body weight height fat amount and body fat percentage (for all $p<0.01$–0.001) No such correlations were seen in the case of prolactin

DISCUSSION

In this study the pubertal development of healthy school children was investigated in a

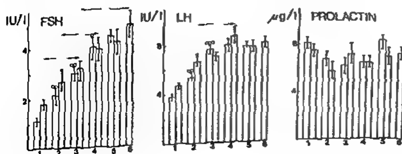


Fig 4 Serum FSH LH and prolactin according to external genitalia (light columns) and pubic hair (dark columns) stages in boys One to three of the symbols O or * indicate $p<0.05$ $p<0.01$ $p<0.001$ significances of the difference in respect to the preceding stage or the stage marked by an arrow

Table 3 *Bone age and serum concentration of FSH and LH according to weight (kg) in girls*

Weight groups	<25	25–29	30–34	35–39	40–44	45–49	50–54	55–59	≥60
Number of subjects	9	16	26	19	21	27	32	21	22
FSH									
Mean	2 0	2 9	5 1	6 5	9 1	9 0	8 2	8 6	9 1
S E M	[illegible] 33	0 58	0 65	0 78	0 60	0 71	0 47	0 74	0 62
LH									
Mean	4 7	5 8	6 9	8 7	1 7	12 2	12 4	12 8	11 6
S E M	0 50	0 45	0 41	0 73	1 68	1 04	0 80	1 53	0 87
Bone age									
Mean	8 0	8 8	10 [illegible]	11 5	13 5	14 [illegible]	15 8	15 8	16 4
S E M	0 76	0 6	0 24	0 30	0 39	0 47	0 77	0 43	0 23

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It is quite well documented that in adults serum prolactin concentration is higher in females than in males (2 21 26) It has also been reported that children have lower prolactin levels than adult females (22) However conflicting data have been reported on serum prolactin levels during puberty In a number of studies no increase in serum prolactin concentration was seen during pubertal development in boys and girls (2 9 19 20) On the contrary Ehara et al (7) found a significant increase in prolactin concentration in girls between 14 and 15 years of age but no changes were seen in boys The prolactin concentration has also been reported to rise steadily with age and pubertal development in girls (3) Possible explanations for the conflicting results include variations in the timing of sam

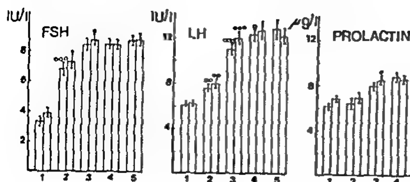


Fig 3 Serum FSH LH and prolactin a cording to breast (light columns) and pub hair (dark columns) stages in girls One three of the symbols O or * indica $p<0.05$ $p<0.01$ $p<0.001$ significances difference in the preceding stage for brea and pubic hair development respectively

lated to pubertal stages in girls using breast development and pubic hair growth as the parameters (23) FSH shows the most significant rise between stages 1 and 2 whereas LH does so between stages 2 and 3 Prolactin shows a small increase between stages 2 and 3 ($p<0.05$) Similarly, Fig 4 demonstrates the hormonal levels in relation to pubertal stages in boys using genital development and pubic hair growth as the parameters It is seen that FSH increases gradually throughout the pubertal stages, whereas LH remains unchanged from genital stage 3 onwards and from pubic hair stage 4 forwards (Fig 4) In contrast to girls serum prolactin demonstrates no change in relation to pubertal stages

Both FSH and LH in girls show a high correlation with chronological age bone age pubic hair and breast developmental stages ($r=0.57$–0.60 for all correlation coefficients $p<0.001$) Prolactin correlates with bone age chronological age and pubertal stage ($p<0.05$)

In prepubertal girls (breast and pubic hair stage 1 bone age <9.5 years) a correlation ($p<0.01$) between LH and body weight as well as calculated fat amount and body fat percentage is seen In this age group LH does no correlate with bone age Longitudinally whe the differences in hormones and physical para meters between the two sample collections a correlated a rise in weight correlates with rise in LH ($p<0.05$) but not with the othe hormones in the whole female population

Table 3 shows the female population grou ed on the basis of weight and for a compariso with Table 1 the mean bone age in the corr sponding weight groups has also been calcu lated An increase in serum LH is seen fro the first group onwards and the difference be tween the first and the third group is signif cant ($p<0.01$)

In boys serum FSH and LH correlates wi chronological age pubic hair stages tot testicular volume body weight height f amount and body fat percentage (for al $p<0.01$–0.001) No such correlations wer seen in the case of prolactin

DISCUSSION

In this study the pubertal development o healthy school children was investigated in

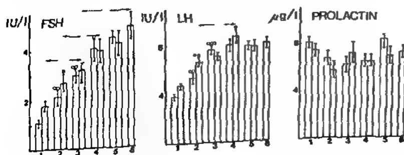


Fig 4 Serum FSH LH and prolactin a cording to external genitalia (light column and pubic hair (dark columns) stages i boys One to three of the symbols O or indicate $p<0.05$ $p<0.01$ $p<0.001$ signifi cances of the difference in respect to th preceding stage or the stage marked by a arrow

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CHILDHOOD CANCER IN SWEDEN 1958–1974

I *Incidence and mortality*

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ABSTRACT Ericsson J L E Karnstrom L and Mattsson B (Central Laboratory for Clinical Pathology University of Uppsala Uppsala and the Stockholm–Gotland Oncologic Centre Karolinska Hospital Stockholm Sweden) Childhood Cancer in Sweden 1958–1974 I Incidence and Mortality Acta Paediatr Scand 67 425 1978.—All cases of tumours and tumour like conditions in children 0–14 years reported to the Swedish cancer registry during the period 1958–74 have been studied The material consists of 3 797 individuals on file in this registry The most common cancer diagnoses in children 0–14 years are leukemia and tumours of the central nervous system (together constituting approximately [redacted] % of all cases) Almost half of the cancers affect children below five years of age The lowest incidence occurs in the ages 7–8 years and the highest during the first year of life The types of tumours below one year of age show a different distribution than in other age groups A significant increase in the incidence of childhood cancer occurred while the mortality rates showed a slight decrease during the period studied A remarkable increase in the incidence figures was noted concerning tumours of the nervous system especially in boys The decrease in the mortality rates was most obvious regarding Wilms tumour and leukemia [redacted] children 0–4 years of age

KEY WORDS Childhood cancer incidence mortality

Recent evidence stresses the importance of the epidemiologic approach to the study of etiologic factors in the causation of cancer in adults as well as in children

Earlier studies from the Swedish cancer registry have revealed a significant increase in the incidence of malignant tumours since the start of the national registration of incidence of cancer in 1958 (1 11) Thus the overall mean annual increases have been 2 3% for males and 1 5% for females while the mortality rates—as revealed by mortality statistics—have been decreasing slightly over recent decades with a decrease of 0 4% annually for males and 0 7% for females

Great variability in the incidence trends of different types of tumours has been noted in Sweden For instance primary cancer of the liver has shown an increase of approximately 7% per year while cancer of the stomach has decreased by about 2 5% annually These results concern the population as a whole Knowing that the pattern of cancer diagnoses in childhood differs very much from that in adults no conclusions concerning childhood cancers can be drawn from the aforementioned studies

It is the purpose of this paper to supply data concerning the incidence and mortality of childhood cancer in Sweden since the inception of the national cancer registry with special reference to analyses of changes of the rates (trend patterns)

MATERIAL AND METHODS

All cases of tumours and tumour like conditions in children 0–14 years reported to the Swedish cancer registry during the period 1958–74 have been studied The material consists of 3 797 individuals on file in this registry In addition to histologically clearly malignant tumours and tumour like conditions—such as leukemias—some histologically benign lesions are included (mainly intracranial and intraspinal meningiomas and neurinomas) (11) For

Table 3 *The total number and frequency of the most common cancers with regard to localization and histopathological type in children 0–14 years 1958–1974*

Diagnosis	Total number 1958–74		Average no /year 1958–62		Average no /year 1963–68		Average no /year 1969–74	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Leukemia								
Chronic myelogenous	28	14	3 4	1 6	0 7	0 7	1 2	0 3
Chronic lymphatic	15	[illegible]	2 0	0 8	0 5	1 0	0 3	0 3
Acute myelogenous	77	63	6 0	4 0	3 5	3 2	4 3	4 0
Acute lymphatic	138	114	3 6	3 2	7 7	6 8	12 3	9 5
Acute blast-stem cell	321	249	16 6	16 4	22 8	16 0	16 8	11 8
Other and unspecified	35	42	3 2	3 2	1 8	2 2	1 3	2 2
Central nervous system								
Astrocytoma oligo-dendroglioma	202	203	9 4	10 6	12 5	12 7	13 3	12 3
Ependymoma	60	62	3 2	4 2	2 8	3 7	4 5	3 2
Medulloblastoma	88	46	3 4	3 0	5 8	2 5	6 0	2 7
Other and unspecified	145	117	7 0	4 8	8 2	6 5	10 2	9 0
Peripheral nerves								
Neuroblastoma	94	70	4 4	3 0	4 8	3 3	7 2	5 8
Neurofibrosarcoma	8	12	0 6	1 2	0 7	1 0	0 2	–
Ganglioneuroblastoma	7	12	0 4	1 0	0 3	0 8	0 5	0 3
Other and unspecified	5	3	0 2	0 2	0 2	0 3	0 5	–
Malignant lymphoma								
Hodgkin	63	27	3 4	1 4	3 0	2 7	4 7	0 7
Histiocytic	39	23	3 2	1 8	2 3	1 2	1 5	1 2
Lymphocytic	69	24	2 2	1 0	4 3	1 0	5 3	2 2
Other and unspecified	63	23	2 8	1 6	3 8	0 8	4 3	1 7
Kidney								
Wilms	114	123	6 6	5 2	5 7	8 5	7 8	7 7
Other and unspecified	7	3	0 6	0 2	–	0 2	0 7	0 2
Eye								
Retinoblastoma	65	52	4 4	2 6	3 3	3 2	3 8	3 3
Other and unspecified	28	39	1 4	1 6	1 2	1 7	2 3	3 5
Bone								
Ewing s sarcoma	25	[illegible]	2 0	0 6	1 7	1 3	0 8	1 5
Osteosarcoma	51	37	2 8	2 6	2 3	1 8	3 8	2 2
Other and unspecified	39	33	3 2	1 4	1 7	1 3	2 2	3 0

and tumour like conditions with regard to localization and histopathological type during the period under study is given in Tables 3 and 4 Table 3 also shows the changes in frequency during three intervals of this period Among the leukemias blast and stem cell forms dominate The most common tumours in the central nervous system are astrocytomas and related forms (spongioblastomas oligodendrogliomas) while neuroblastomas predominate in the peripheral nervous system

Teratoma is a comparatively common histopathological diagnosis in children (Table 4)

Age distribution

Almost half of the cancers affect children below 5 years of age (Fig 1) This is most conspicuous regarding leukemia Wilms tumour and neuroblastoma Leukemias show a peak between the age of 2 and 4 years The lowest incidence is noted in the ages 7 to 8 years Then a slight increase occurs with increasing

Table 1 . Annual mean population cancer cases deaths and deaths from cancer in children 0–14 years 1958–74

Sex	No of children in population	Cancers		Deaths		Cancer deaths	
		No	/10⁶	No	/10⁶	No	/10⁶
Boys	853 454	123	142	1 225	1 435	79	93
Girls	807 912	101	124	861	1 066	62	77

convenience of presentation all tumours and tumour like conditions which are to be reported to the registry and are included in the incidence figures have been referred to as cancers in the following

Histological verification of the lesions was obtained in approximately 95% of the cases Mortality figures have been obtained from the files of the National Central Bureau of Statistics (12) For the evaluation of incidence and mortality trends a linear regression analysis based on the observed rates per one million population has been performed The statistical significance of the trends has been checked by t test

The linear regression equation used was of the form

$$Y_e = a + bX$$

where

Y_e = estimated annual incidence rate per one million population

Y = observed annual incidence rate per one million population

X = calendar year minus 1957 for death rates 1959

$a = \bar{Y} - b\bar{X}$

and

$$b = \frac{\Sigma(X-\bar{X})(Y-\bar{Y})}{\Sigma(X-\bar{X})^2}$$

The regression coefficient b has been t tested with 15 degrees of freedom for incidence rates and 13 degrees of freedom for mortality figures

RESULTS

In Sweden the average number of new cancer cases in children amounts to approximately 220 annually Table 1 shows the annual mean population at risk number of primary cancer cases and number of deaths from cancer It will be seen that such tumours are somewhat more common in boys than in girls The most common diagnoses in children are leukemia and tumours of the nervous system which together constitute 58% of the total A summary of the ten most common cancer sites is given in Table 2 None of the most common childhood cancers are frequent in adults Hence leukemias account for about 3% and tumours of the brain and spinal cord 3 5% among adults Furthermore Wilms tumour of the kidney and neuroblastomas are extremely rare among grown ups

The frequency of the most common tumours

Table 2 *The ten most common cancer sites in children 0–14 years in each sex 1958–74*

Site	No	%
Boys		
1 Leukemia	614	30
2 Nervous system	581	28
3 Malignant non Hodgkin lymphoma	152	7
4 Kidney	121	6
5 Bone	115	6
6 Eye	93	4
7 Connective tissue muscle	66	3
8 Hodgkin s disease	63	3
9 Endocrine glands (except thyroid gland)	51	2
10 Testis	48	2
Other sites	182	9
Total	2 086	100
Girls		
1 Nervous system	504	29
2 Leukemia	494	29
3 Kidney	126	7
4 Eye	91	5
5 Bone	90	5
6 Connective tissue muscle	61	4
7 Malignant non Hodgkin lymphoma	60	4
8 Ovary	36	2
9 Colon (except rectum)	32	2
10 Endocrine glands (except thyroid gland)	31	2
Other sites	186	11
Total	1 711	100

Table 5 *Number and per cent distribution of cases diagnosed before the age of one for a selected group of sites*

Site	Boys		Girls	
	No 0–1 year	Per cent of total 0–14 years	No 0–1 year	Per cent of total 0–14 years
Liver	12	44	10	47
Vulva vagina	–	–	5	56
Testis	11	23	–	–
Eye	26	78	21	23
Endocrine glands (except thyroid gland)	10	20	4	13
Connective tissue muscle	11	17	13	21
Reticuloses and related forms of malignant lymphoma	11	58	4	40

common cancers have been studied separately For the largest group—leukemia—there is an approximately constant incidence during the whole period (Fig 3) while a significant decrease in the mortality rates is noted for girls

Looking at the three age groups 0–4 5–9 and 10–14 years separately remarkable differences are noted in the trends especially for mortality (Figs 4–6) In the age group 0–4 years there is a significant decrease in the death rates while the incidence rates show a slight increase (Fig 4) In the following age group (5–9 years) no corresponding change in the mortality rates appear On the contrary there is an increase for boys Furthermore death rates for both boys and girls are higher than the incidence rates In the oldest age group studied (10–14 years) there is a tendency to an overall decrease both in the mortality and incidence rates Again mortality rates exceed the incidence rates at the end of the period

For figures regarding the different subgroups of leukemia reference is made to Table 6

In order to be able to compare the rates of incidence and mortality for tumours of the

Table 6 *Mean annual change in incidence and mortality for different sites in children 0–14 years 1958–74*

Site		0–4		5–9		10–14		0–14	
		M	F	M	F	M	F	M	F
All sites	incid	5	2 0	7	–1 0	1 5	0 1	2 3	0 7
	mort	– 3	–2 3	3 1	–0 3	–0 8	–1 9	–0 6	–1 5
Leukemia	incid	1	2 2	3 3	–1 4	–2 4	–1 8	0 8	–0 5
	mort	–3 8	–4 4	6 4	–0 4	–1 5	–1 0	–0 8	–2 2
Nervous system	incid	11 9	7 0	2 3	–2 3	3 0	8	6 1	2 [illegible]
	mort	4 3	1 1	2 3	–1 3	0 1	–1 1	7 5	–0 3
Central nervous system	incid							4 1	1 0
Neuroblastoma	incid							3 4	11 9
Wilms tumour	incid							1 [illegible]	2 4
	mort							–4 7	–3 7

$p<0.05$ $p<0.01$ $p<0.001$

Table 4 *The total number of selected histopathological types in children 0–14 years 1958–1974*

Diagnosis	Total number 1958–74 Boys	Girls
Other selected histological types		
Granulosa—theca cell tumour of ovary		11
Teratoma testis	35	
Malignant teratoma (excl testis)	15	31
Adenoma of endocrine glands	7	3
Rhabdomyosarcoma	22	10
Fibrosarcoma	17	18
Angiosarcoma	11	9
Adenocarcinoma	19	51
Squamous carcinoma	3	1
Carcinoma unspecified	8	5
Malignant mesenchymal tumour	27	20
Carcinoid	8	29
Malignant melanoma (excl skin)		3

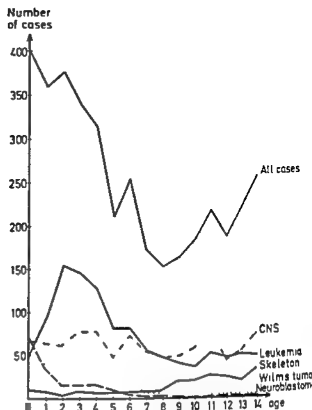


Fig 1 Age distribution at the time of diagnosis for all cases and selected large groups Total number 1958–74

age dependent on the addition of other forms of tumours for example those of the skeleton (Fig 1) A slightly higher incidence is shown for boys than for girls for all ages (Table 1)

The sites and cancer forms illustrated in Fig 1 do not account for the high incidence in the age group 0–1 year Analysis of this group shows that a number of special tumour forms occur very frequently during the first year of life (Table 5) For instance slightly over 40% of all malignant tumours of the liver in children were diagnosed in this group Other malignant tumours with an early predominance include reticuloses and related forms of malignant lymphomas and malignant tumours of the vulval vaginal sites

Trends in incidence and mortality

The trends in incidence and mortality for all cancers during the period 1958–74—as revealed by linear regression analysis—are shown in Fig 2 A significant increase in the incidence rates is noted for boys (2 3% annually) There is also an increase for girls although considerably less significant (0 7% annually) The mortality rates during the corresponding period show a decrease which is most remarkable for girls Table 6 shows the average annual change in per cent The trends in incidence and mortality for the three most

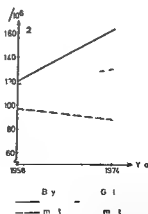


Fig 2 Trends in incidence and mortality for all cancers in the age group 0–14 years during the period 1958–74 as revealed by linear regression analysis

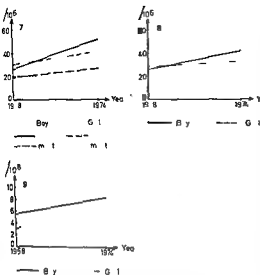


Fig 7 Trends in incidence and mortality for tumours of the nervous system (central and peripheral) in the age group 0–14 years
Fig 8 Trends in incidence for tumours of the central nervous system in the age group 0–14 years
Fig 9 Trends in incidence for neuroblastomas in the age group 0–14 years

These include primary tumours of the liver (mainly hepatocellular carcinomas but also angiosarcomas and malignant teratomas) (4) vulvo-vaginal tumours and reticuloses This very early predominance of the aforementioned uncommon tumours in children has not been demonstrated earlier Nothing is presently known about possible etiologic factors for these tumours The parents have so far not been studied with regard to occupation exposure to carcinogens or other agents In a study from the Finnish cancer registry no significant correlations were found between potential etiologic factors and the cases of cancer (7) and no significant association was noted between the commonest types of childhood cancer and hydrocarbon related occupations among the fathers (3)

The incidence peak for leukemia (2–3 years) is for unknown reasons somewhat earlier than in other materials (8–10) Increasing incidence trends were noted for the whole population studied and also for certain types of tumours while other types showed a decrease Trends for the same tumour also differed between the age groups This indicates that the increasing trends observed for some of the tumour types are not likely to be due to improved methods of diagnosis or change in diagnostic criteria during the later years The fact that certain types of tumours show increased incidence rates over the years while others tend to decrease does not support the notion that a more careful reporting to the cancer registry has occurred during later years Furthermore the trends appear to be similar in other Scandinavian countries The frequency of cancers diagnosed accidentally at autopsy is extremely low and possible changes in this frequency over the years can not affect incidence figures in such a way that increasing trends can occur

The major part of the increase of incidence rates in Sweden is accounted for by tumours of the nervous system If these tumours are excluded the tendency toward an augmented occurrence of childhood cancer is abolished It is interesting to note that the increase in tumours of the nervous system in children is about three times higher in boys than in girls Similar figures have been reported from Finland and Norway (9 13) No corresponding trend is noted in adults If environmental factors were of importance for the development of these tumours in children no sex difference regarding the trends should occur

The decrease in death rates in the age group 0–4 years for leukemia is almost certainly explained by improved methods of treatment

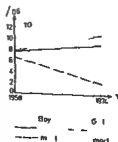


Fig 10 Trends in incidence and mortality for Wilms tumour in the age group 0–14 years

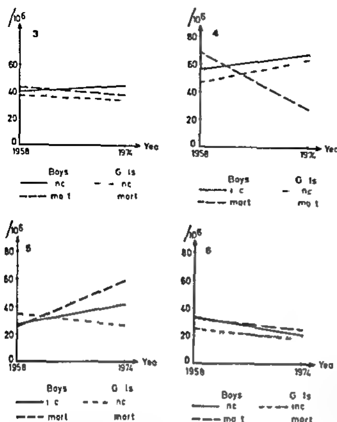


Fig 3 Trends in incidence and mortality for leukemia in the age group 0–14 years
Fig 4 Trends in incidence and mortality for leukemia in the age group 0–4 years
Fig 5 Trends in incidence and mortality for leukemia in the age group 5–9 years
Fig 6 Trends in incidence and mortality for leukemia in the age group 10–14 years

nervous system, peripheral and central nervous system tumours had to be grouped together (no subdivision is achievable concerning death rates) (12) As illustrated in Fig 7, a significant increase in the incidence is noted for both sexes The same holds true for boys regarding the mortality while the mortality rates for girls are fairly constant The increase in the incidence concerns both tumours of the central nervous system and the most common type in the peripheral nervous system —neuroblastomas (2) (Figs 8 and 9)

The majority of the cases of Wilms tumour occurred before the age of 6 The incidence has remained largely unchanged between 1958 and 1974 However a remarkable decrease in the mortality is noted in both sexes (for boys 4 7% annually, and for girls 3 7%) (Fig 10)

DISCUSSION

In comparison with the rate of cancer in the whole population, the number of tumours in children is low (11) However childhood cancer ranks third among causes of death after perinatal deaths and accidents (12)

The present study is based entirely on the reports as they appear in the Swedish national cancer registry This means that there is a certain underestimation of the incidence figures in the registry due to the fact that cases known only by death certificates are not included in the registry Hence a recent study of the completeness of registration in the cancer registry (5) has shown that about 3–4% of the diagnosed cancer cases were not reported Certain diagnoses—among them leukemias—were overrepresented in this loss In general the diagnoses have not been checked but are based on the primary reports from the clinician and the pathologist

The rates for all cancer in children are well in line with reports regarding the incidence reported in other studies from the Western hemisphere (4 5 6 7) These reports also show that the majority of the cases include leukemias and tumours of the nervous system Other more uncommon tumours also seem to show approximately the same frequency as in other reports (6 8 9 10) although most of these have not gone into detail concerning histopathological types

With regard to the age distribution of the tumour cases in Sweden a predominance was noted during the first 5 years of life However considerable variation occurs depending on the type of tumour Thus as in Finland (9) neuroblastomas Wilms tumour and retinoblastomas (embryonal tumours) show a high incidence during the first years of life while tumours of the central nervous system are evenly distributed over the years and skeletal tumours predominate during the late phase of the age span studied In Sweden certain uncommon tumours were found to occur with high frequency during the first year of life

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Methods have been developed that eliminate these difficulties either by substituting the saline load for hypertonic mannitol solutions (1 20 23) or by reducing the period of hydric restriction (10 14) Nevertheless all procedures insist on 10-hour periods of fluid restriction which is still badly tolerated by infants affected by a disturbance of the renal concentration mechanism

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In a previous study (17) the results obtained in a pediatric population were evaluated with a modified technique of mannitol infusion method The previous period of hydropenia and the loss of sodium and potassium induced by the mannitol were eliminated

This paper refers to the results obtained

This view is supported by the observed increase in mortality rates in the age group 5–9 years Indeed, in this age group rates for mortality exceed those of incidence Thus it would seem that more sophisticated methods of treatment in most cases only have resulted in prolonged survival up to the year 1974 However in the case of Wilms tumour mortality rates have decreased remarkably evidently due to improved treatment Lowered mortality rates are on the other hand not observed with regard to tumours of the nervous system

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with this technique at different pediatric ages The usefulness of this method is considered on differential diagnosis of children affected by pituitary and nephrogenic diabetes insipidus and the appraisal of different oral drugs used in its treatment

PATIENTS

Forty patients sent to the Paediatric Nephrology Unit for screening of renal or pituitary diseases were studied Through regular clinico-biochemical and radiological methods pyelonephritis or complex tubulopathies were discarded No case showed clinical or bacteriological signs of urinary tract infection the last two months prior to the presented studies

Group I (control) includes 31 patients The reasons for consultation were basically enuresis in children and suspicion of tubular or pituitary disease in infants In no case did there appear nephrological or endocrine abnormalities For better appraisal of results the group has been divided according to age groups IA 2–6 months IB 6–12 months and IC 1–7 years

Group II formed by six patients with polyuria–polydipsia syndrome considered as pitressin sensitive or pituitary diabetes insipidus (PDI) In two cases (A B C and R B R) there was no sign of cerebral injury or tumor being considered as 'idiopathic' forms of PDI In the four remaining patients there was an infectious or underlying tumor cause capable of explaining the endogenous defect of vasopressin In each case the study was performed after stopping the administration of exogenous vasopressin seven days prior

Group III includes three patients with polyuric syndrome pitressin resistant The studies to discard pyelonephritis or complex tubular pathology were negative They had been clinically diagnosed as nephrogenic diabetes insipidus (NDI) The renal histological study of the three patients showed a normal parenchyma

In all cases the parents were fully informed and written consent was always obtained The parents were also kept informed of the clinical evolution of the child

METHODS

The patients were studied in the morning without any fluid intake restriction Thus the individual fluid balance was not changed by any preparative measure The patients remained in our metabolic ward for the two hour duration of the test Two indwelling needles were placed into peripheral veins one for infusion of the test substances one for repeated blood sampling For urine sampling a polyethylene catheter was used The complete vesical voiding after each period of urine collection was achieved by air flushing Blood and urine samples were withdrawn for blank values according to standard clearance technique

After a priming dose of 1 ml/kg b w of 10% inulin a continuous infusion able to maintain plasmatic levels of 50 mg/100 ml throughout the test was held The infusion rate was 1 ml/min/m² After an equilibration time of 45 min the test developed in two phases In the first the infusion was replaced by a mannitol infusion containing mannitol 10% in isotonic saline inulin as mentioned and potassium calculated to deliver 5 mEq/m²/h The infusion rate was set at 6 ml/min/m² During mannitol infusion 4-5 urine samples were collected at 5–10 min intervals Blood samples were obtained every 15 min In the second phase vasopressin in aqueous solution (Pitressin Parke Davis) was added to the otherwise unchanged infusion in amounts calculated to deliver 8 mU/h/kg b w The infusion rate and the timed collection of urine and blood samples were similar to those stated above

Blood pressure was controlled during the study The fluid balance was continuously maintained Water supplements were given orally when urinary output exceeded intake

Therapeutic study with carbamazepine and clofibrate Four patients with PDI received carbamazepine 600 mg/day for a week Osmolar clearance test was performed prior to the beginning of and on the last day of administration of the drug

Two patients with idiopathic PDI successively received 600 mg daily of carbamazepine 1 500 mg daily of clofibrate and the association of both drugs all in periods of a week A week without any medication was allowed between each cycle of the corresponding drug The osmolar clearance study was done on the last day of administration of each of the drugs or combination of them

Calculations Serum concentrations at the midpoint of each urine sample were read from a concentration/time graph Osmolar clearance (C_{osm}) defined as the total amount of osmotically active substance cleared is expressed as every clearance

$$C_{osm} = \frac{U_{osm} V}{P_{osm}}$$

If $U_{osm} > P_{osm}$ $C_{osm} > V$ $C_{osm} - V = T_{H_2O}$

The free water reabsorption (T_{H_2O}) is defined as the volume of water reabsorbed over an equivalent amount of solutes with which it would be isoosmotic

If $U_{osm} < P_{osm}$ $C_{osm} < V$ $V - C_{osm} = C_{H_2O}$ $C_{H_2O} = -T_{H_2O}$

The free water excretion or free water clearance (C_{H_2O}) represents the volume of water eliminated over an equivalent amount of solutes with which it would be isoosmotic

A negative value of T_{H_2O} is equivalent to a positive value of C_{H_2O} If $U_{osm} = P_{osm}$ $C_{osm} = V$ The urine is isoosmotic to the plasma There is neither reabsorption nor excretion of osmotically free water

The values of T_{H_2O} and C_{H_2O} were evaluated in relationship with C_{osm} which originated them

Analytical procedures Inulin in plasma and urine was determined according to the anthron method of Davidson & Sackner (6) Osmolality was estimated by the reduction

Table 1 *Representative clearance study in a normal infant (D G M 3 months)*

Time (min)	ml/min/1 73 m C_{in}	ml/min/1 73 m C_{PAH}	mOsm/kg P_{osm}	mOsm/kg U_{osm}	ml/100 ml GFR V	ml/100 ml GFR C_{osm}	ml/100 ml GFR T_{H_2O}
0	Priming injection of inulin and PAH Sustaining infusion with inulin and PAH infusion rate 1 ml/min/m²						
47	Infusion I Mannitol 10% in saline 0 9% potassium chloride inulin PAH infusion rate 6 ml/min/m²						
47– 61	58	762	280	850	0 92	2 80	1 88
61– 66	106	471	283	757	1 64	4 35	2 71
66– 71	90	405	84	674	2 55	6 06	3 51
71– 76	93	439	86	674	3 71	8 10	4 39
76– 81	69	359	288	583	4 99	10 09	5 10
81	Infusion II Mannitol 10% in saline 0 9% potassium chloride inulin PAH vasopressin infusion rate 6 ml/min/m²						
81– 86	57	296	289	552	6 48	12 38	5 90
86– 91	60	332	291	550	7 62	14 41	6 79
91– 96	63	330	293	574	10 13	18 12	7 99
96–101	[illegible]	330	293	519	9 38	16 62	7 24
101–106	61	333	293	548	12 37	23 13	10 76

of freezing point on an Advanced Osmometer 3D The sodium and potassium concentration were read on an IL 343 photometer and chloride on an IL 279

Statistical method The dispersion chart which relates T_{H_2O} with C_{osm} in the control group was compiled with all the values obtained from the 31 cases The adjustment curve used was a geometrical curve with the equation $y=ax^b$ (9) applying two standard deviations by the method of minimus squared Through logarithmic conversion the problem can be solved as a linear regression To compare the three control subgroups among themselves the values of T_{H_2O} were analyzed within each of seven C_{osm} ranges 2–4 ml 4–6 ml 6–8 ml 8–10 ml 10–12 ml 12–14 ml 14–16 ml When a particular case showed more than one value within the mentioned range the arithmetic average of the values of C_{osm} and their corresponding values of T_{H_2O} were obtained These data—one per case for each range of C_{osm}—were analyzed (Table 3) The values of T_{H_2O} were compared among the three subgroups as long as the difference between the values of C_{osm} within each range was not statistically significant

The statistical significance of the data in the control group was analyzed by means of Student s and Cochran s tests for matching data and observed averages (19)

Table 2 *Means and SD values of the plasma sodium potassium chloride and osmolality on the first (F) and late (L) period of osmolar clearance study in 31 normal infants and children of the control group*

Group	Age (range)	C_{in} ml/min 1 73 m	P_{Na} mEq/l F	P_{Na} mEq/l L	P_K mEq/l F	P_K mEq/l L	P_{Cl} mEq/l F	P_{Cl} mEq/l L	P_{osm} mEq/l F	P_{osm} mEq/l L
1A (N=6)	2–5 m	62 (24)	138 (2 8)	135 (3 7)	4 5 (0 6)	4 7 (0 7)	101 (3 1)	100 (6)	285 (5 8)	293 (7)
1B (N=5)	6–11 m	98 (26)	142 (4 3)	138 (5 5)	4 9 (0 2)	4 8 (0 2)	–	–	293 (4 9)	301 (3 9)
1C (N= 0)	1–7 y	120 (17)	139 (3 2)	135 (4)	4 3 (0 4)	4 4 (0 5)	104 (4)	104 (5)	285 (4 4)	293 (5 5)
Statistical analysis of the individual values in the 31 cases										
M ±S D			140 3 4	136 4 [illegible]	4 4 0 5	4 5 0 5	104 3 8	103 5 2	286 5 4	294 6 1
p (paired series)			<0 0001		NS		NS		<0 0001	

NS = Not significant

Table 3 *Osmolar clearance (C_{osm}) and T_{H_2O} at various ranges of C_{osm} in normal patients (Group I)*
$M \pm S D$ = mean ± 1 S D of C_{osm} and T_{H_2O} were determined for all points within each range of the age groups

Group		2–4		4–6		6–8		8–10		10–12	
		C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}
Range of C_{osm} (ml/min/1 73 m²)											
I A (N=6)	M	2 90	1 70	5 36	2 94	7 03	3 45	8 83	3 95	10 81	4 5
	±S D	0 61	0 42	0 48	0 42	0 59	0 38	0 55	0 30	0 49	0 7
		(N=4)		(N=5)		(N=6)		(N=5)		(N=6)	
I B (N=5)	M	2 80	1 91	5 21	3 16	6 95	3 64	8 93	4 46	10 51	4 98
	±S D	0 35	0 11	0 49	0 30	0 58	0 14	0 37	0 19	0 13	0 56
		(N=3)		(N=5)		(N=4)		(N=4)		(N=7)	
I C (N=20)	M	3 04	2 09	5 22	3 16	7 04	3 95	9 10	4 64	11 07	5 79
	±S D	0 50	0 39	0 64	0 61	0 49	0 48	0 46	0 39	0 39	0 40
		(N=11)		(N=11)		(N=12)		(N=15)		(N=16)	
Statistical analysis											
I C–I A	p	NS	NS	NS	NS	NS	<0 05	NS	<0 005	NS	<0
I C–I B	p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Range of C_{osm} (ml/100/GFR)											
I A (N=6)	M	3 06	1 86	5 04	2 74	6 85	3 49	8 70	4 06	10 53	4 8
	±S D	0 44	0 25	0 62	0 24	0 70	0 24	0 48	0 35	0 56	0 4
		(N=6)		(N=5)		(N=5)		(N=6)		(N=4)	
I B (N=5)	M	2 80	1 93	5 24	3 08	6 70	3 53	8 09	4 15	11 35	5 5
	±S D	0 23	0 18	0 45	0 16	0 64	0 40			0 41	0 3
		(N=3)		(N=4)		(N=4)				(N=3)	
I C (N=20)	M	2 76	1 72	4 88	2 70	7	3 42	8 73	3 99	10 69	4 6
	±S D	0 47	0 23	0 64	0 45	0 20	0 30	0 63	0 45	0 50	0 3
		(N=16)		(N=15)		(N=17)		(N=14)		(N=11)	
Statistical analysis											
I C–I A	p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
I C–I B	p	NS	NS	NS	NS	NS	NS	NS	NS	=0 05	

p =Differences in means between groups for each range were examined for significance using standard variance analy
NS=Not significant

RESULTS

A representative example of the study is shown in Table 1 The variations observed in the glomerular filtration rate (GFR) and renal plasma flow (RPF) secondary to the manni tol load are proportionate for both para meters (1)

Group I (control) Plasma concentrations of potassium and chloride prior to and at the end of the study did not show significant dif ferences (Table 2) There is a highly significant tendency to hyponatremia ($p<0\ 0001$) but the average plasmatic concentration of sodium at the end of the test (136±4 2 mEq/l) must be considered on the lower borderline of nor mality Plasma osmolality shows a significant increase ($p<0\ 0001$) induced by the hyper tonic mannitol overload

The analysis between the average values of C_{osm} of the different subgroups does not show any significant differences in any of its dif ferent ranges It allowed determination of the statistical significance of the respective values of T_{H_2O} (Table 3) The results were analyzed by comparing the subgroups of the younger children (I A and I B) with the children over one year old (I C)

According to 1 73 m² b s at low levels of C_{osm} the differences of T_{H_2O} were not signifi cant Above a C_{osm} of 6 ml/min/1 73 m² b s the differences of T_{H_2O} between groups I A and I C are significant ($p<0\ 05$) progressively

?–14		14–16	
C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}
? 91	4 87	15 45	5 57
0 47	0 5?		
(N=?)			
? 76	5 5?	14 8?	5 97
		0 38	0 18
		(N=?)	
? 67	5 8	14 74	6 63
0 56	0 64	0 46	0 64
(N=17)		(N=14)	
NS	=0 05		
NS	NS	NS	NS
1? 80	5 ‿6	14 87	5 9?
0 34	0 56	0 36	0 63
(N=5)		(N=4)	
13 15	5 9?		
0 49	0 ??		
(N=8)			
1? 87	5 73	14 56	5 99
0 47	0 49	0 18	0 91
(N=8)		(N=6)	
NS	NS	NS	NS
NS	NS		

increasing ($p<0.0005$) according to the increase of C_{osm}. The comparison of the T_{H_2O} between groups I B and I C did not show any significant differences at any level of C_{osm} (Table 3).

Related to 100 ml GFR the data showed no significant differences at any range of C_{osm} among the established age subgroups.

The dispersion chart of all the values of T_{H_2O} plotted against C_{osm} allowed calculation of the adjustment curve $y=0.80x^{0.75}$ $r=0.98$ ($p<0.0001$) (Fig. 1). The application of two standard deviations identifies the area of normality according to the sample of the control group. A similar adjustment curve referring to 1.73 m² b.s. is expressed by the equation $y=$ 11.88$x^{0.75}$ $r=0.97$ ($p<0.0001$). It includes only the cases of children over six months old whose statistical differences were not significant.

Group II. Pituitary Diabetes Insipidus (PDI). In Fig. 2A the patients are referred to differentiating the idiopathic forms from those secondary to underlying pathology. The values of C_{H_2O} and T_{H_2O} reached in the first (open signs) and second (closed signs) phase of osmolar clearance respectively are shown. In this way we investigate the response of neuroendocrine and renal systems in the presence of an osmotic load. In one of the secondary PDI patients (O) although the values of T_{H_2O} are below normal they show up positive before the infusion of vasopressin showing a partial secretion of ADH (Fig. 2A). The infusion of exogenous vasopressin induced a sudden increase of T_{H_2O} in all the cases.

Group III. Nephrogenic Diabetes Insipidus (NDI). The results are presented in Fig. 2B. In spite of the infusion of vasopressin the values of C_{H_2O} increased in relation to the osmotic load.

Action of carbamazepine on PDI. The results of osmolar clearance obtained in four patients with PDI before and after the treatment with carbamazepine are shown in Table 4. In order to obtain a better appraisal of the effect of the drug periods with a similar C_{osm} have been compared. The GFR was not significantly modified between both studies of each patient. During the mannitol phase (M) the reduction of C_{H_2O} after carbamazepine was evident in three of the four patients (A. B. C., R. B. G. and J. B. R.) values of T_{H_2O} without vasopressin addition.

Action of clofibrate and carbamazepine on PDI. In those patients with idiopathic PDI (R. B. R. and A. B. C.) the results of osmolar clearance before and after the administration of clofibrate, carbamazepine and both drugs together were compared (Table 5).

The C_{In} prior to each osmotic overload did not show any differences that would indicate any action of the drugs on the GFR. The in-

Table 3 *Osmolar clearance (C_{osm}) and T_{H_2O} at various ranges of C_{osm} in normal patients (Grou I)*
$M\pm$S D = mean ± 1 S D of C_{osm} and T_{H_2O} were determined for all points within each range of the age groups

Group		2–4		4–6		6–8		8–10		10–1[illegible]	
		C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}	C_{osm}	T_{H_2O}
Range of C_{osm} (ml/min/1 73 m²)											
I A (*N*=6)	*M*	2 90	1 70	5 36	2 94	7 03	3 45	8 83	3 95	10 81	4 [illegible]
	±S D	0 61	0 42	0 48	0 42	0 59	0 38	0 55	0 30	0 49	0 [illegible]
		(*N*=4)		(*N*=5)		(*N*=6)		(*N*=5)		(*N*=6)	
I B (*N*=5)	*M*	2 60	1 91	5 21	3 16	6 95	3 64	8 93	4 46	10 51	4 98
	±S D	0 35	0 11	0 49	0 30	0 58	0 14	0 37	0 19	0 13	0 96
		(*N*=3)		(*N*=5)		(*N*=4)		(*N*=4)		(*N*=[illegible])	
I C (*N*=20)	*M*	3 04	2 09	5 22	3 16	7 04	3 95	9 10	4 64	11 07	[illegible]
	±S D	0 50	0 39	0 64	0 61	0 49	0 48	0 46	0 39	0 39	0 40
		(*N*=11)		(*N*=11)		(*N*=12)		(*N*=15)		(*N*=16)	
Statistical analysis											
I C–I A	*p*	NS	NS	NS	NS	NS	<0 05	NS	<0 005	NS	<0
I C–I B	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Range of C_{osm} (ml/100/GFR)											
I A (*N*=6)	*M*	3 06	1 86	5 04	2 74	6 85	3 49	8 70	4 06	10 53	4 8[illegible]
	±S D	0 44	0 25	0 62	0 24	0 70	0 24	0 49	0 35	0 56	0 4[illegible]
		(*N*=6)		(*N*=5)		(*N*=5)		(*N*=6)		(*N*=4)	
I B (*N*=5)	*M*	2 80	1 93	5 24	3 08	6 70	3 53	8 09	4 15	11 35	5 5[illegible]
	±S D	0 23	0 18	0 45	0 16	0 64	0 40			0 41	0 [illegible]
		(*N*=3)		(*N*=4)		(*N*=4)				(*N*=3)	
I C (*N*=20)	*M*	2 76	1 72	4 88	2 70	7	3 42	8 73	3 99	10 69	4 6[illegible]
	±S D	0 47	0 23	0 64	0 45	0 20	0 30	0 63	0 45	0 50	0 3[illegible]
		(*N*=16)		(*N*=15)		(*N*=17)		(*N*=14)		(*N*=11)	
Statistical analysis											
I C–I A	*p*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
I C–I B	*p*	NS	NS	NS	NS	NS	NS	NS	NS	=0 05	

p = Differences in means between groups for each range were examined for significance using standard variance analy
NS = Not significant

RESULTS

A representative example of the study is shown in Table 1 The variations observed in the glomerular filtration rate (GFR) and renal plasma flow (RPF) secondary to the mannitol load are proportionate for both parameters (1)

Group I (control) Plasma concentrations of potassium and chloride prior to and at the end of the study, did not show significant differences (Table 2) There is a highly significant tendency to hyponatremia ($p<0$ 0001) but the average plasmatic concentration of sodium at the end of the test (136±4 2 mEq/l) must be considered on the lower borderline of normality Plasma osmolality shows a significant increase ($p<0$ 0001) induced by the hypertonic mannitol overload

The analysis between the average values of C_{osm} of the different subgroups does not show any significant differences in any of its different ranges It allowed determination of the statistical significance of the respective values of T_{H_2O} (Table 3) The results were analyzed by comparing the subgroups of the younger children (I A and I B) with the children over one year old (I C)

According to 1 73 m² b s at low levels of C_{osm} the differences of T_{H_2O} were not significant Above a C_{osm} of 6 ml/min/1 73 m² b s the differences of T_{H_2O} between groups I A and I C are significant ($p<0$ 05) progressively

Table 4 *Effect of carbamazepine on osmolar clearance in PDI patients*
M=infusion I with 10% mannitol in isotonic saline M+V=infusion II with 10% mannitol in isotonic saline+vasopressin

		Basal ml/min/1.73				Carbamazepine ml/min/1.73			
		V	C_{osm}	T_{H_2O}	C_{H_2O}	V	C_{osm}	T_{H_2O}	C_{H_2O}
A B C (idiopathic PDI)	M	8.88	6.97	–	1.90	7.76	6.93	–	0.33
	M+V	5.76	9.54	3.78	–	8.97	12.25	3.78	–
R B R (idiopathic PDI)	M	24.25	10.55	–	13.70	20.56	10.37	–	10.19
	M+V	11.48	15.52	4.04	–	13.38	15.97	2.59	–
R B G (histiocytosis X)	M	23.23	8.98	–	14.25	9.04	8.45	–	0.58
	M+V	7.23	10.96	3.73	–	6.45	10.57	4.12	–
J B R (optic chiasm glioma)	M	15.04	11.14	–	3.90	8.27	11.32	3.05	–
	M+V	15.04	15.70	0.66	–	9.40	15.10	5.70	–

Similar periods of C_{osm} chosen in each of the patients on the basal studies and after carbamazepine administration

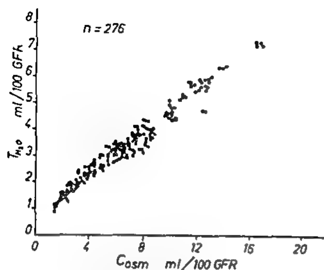
DISCUSSION

High concentrations of mannitol in extracellular fluid induces modifications in the water distribution among body compartments. It can be used for the study of endocrine and renal functions related with the urine concentration mechanism. As with hypertonic saline solutions, with the concentrated mannitol we try to maximally stimulate the functions that influence the renal capacity of free

Table 5 *Effect of carbamazepine and clofibrate on osmolar clearance studied during mannitol diuresis prior to and after vasopressin infusion in two patients with pituitary diabetes insipidus*
M=infusion I with 10% mannitol in isotonic saline M+V=infusion II with 10% mannitol in isotonic saline+vasopressin

		C_{in} (ml/min/1.73)	V (ml/min)	U_{osm}/P_{osm}	ml/min/1.73 m²			ml/100 GFR		
					C_{osm}	T_{H_2O}	C_{H_2O}	C_{osm}	T_{H_2O}	C_{H_2O}
Patient R B R (idiopathic PDI)										
Basal	M	173	15.60	0.46	11.50	–	13.72	9.47	–	11.24
	M+V		7.10	1.35	15.52	4.04	–	15.02	3.91	–
Clofibrate	M	111	16	0.44	11.26	–	14.61	9.11	–	11.83
	M+V		8.80	1.13	16.17	1.89	–	15.07	1.77	–
Carbamazepine	M	131	14	0.51	11.67	–	11.23	8.99	–	8.68
	M+V		8.2	1.19	15.97	2.59	–	12.96	2.10	–
Carb + clof	M	140	15.[illegible]	0.47	10.50	–	14.31	6.10	–	8.31
	M+V		8.40	1.13	15.47	1.76	–	9.89	1.13	–
Patient A B C (familiar idiopathic PDI)										
Basal	M	101	4	0.79	6.97	–	1.90	5.97	–	1.61
	M+V		2.6	1.66	9.54	3.78	–	8.26	3.27	–
Clofibrate	M	115	4.1	0.77	6.49	–	1.95	4.64	–	1.39
	M+V		3	1.60	9.87	3.69	–	9.68	3.67	–
Carbamazepine	M	118	3.4	0.95	6.93	–	0.33	4.64	–	0.22
	M+V		4.7	1.37	17.25	3.28	–	13.20	3.53	–
Carb + clof	M	176	2	1.63	6.97	2.70	–	3.82	1.48	–
	M+V		2.8	1.65	9.87	3.89	–	8.67	3.40	–

Similar periods of C_{osm} in each of the patients on the basal studies and after the administration of carbamazepine, clofibrate and both drugs



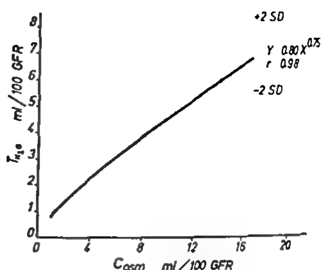


Fig 1 Relationship between fractional free water reabsorption (T_{H_2O}) and osmolar clearance (C_{osm}) in normal patients (Group I) Values obtained under mannitol infusion before (open symbols) and after (closed symbols) vasopressin addition

fluence on the renal concentration capacity was different in both patients In R B R there was no significant difference among the results of all the tests In A B C with familiar idiopathic PDI the clofibrate showed no effect but the carbamazepine induced a pronounced decrease of C_{H_2O} at similar levels of C_{osm} The therapeutic combination of both drugs induced positive values of T_{H_2O} through nearly all the study even before adding vasopressin to the mannitol infusion (Fig 3)

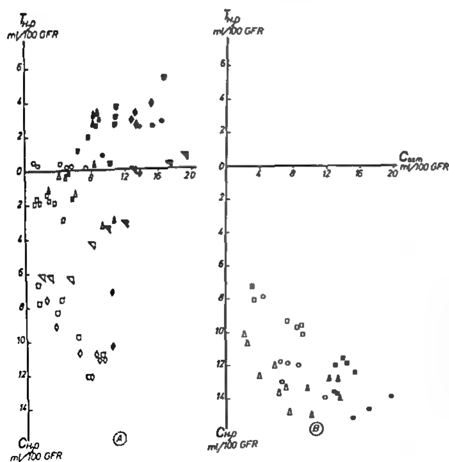


Fig 2 Relationship between fractional free water reabsorption (T_{H_2O}) or free water clearance (C_{H_2O}) and osmolar clearance (C_{osm}) in A) Primary PDI (△◇) and secondary PDI (○□▽◡) patients B) NDI (△○□) patients Values obtained under mannitol infusion before (open symbols) and after (closed symbols) vasopressin addition Shaded area corresponds to adjustment curve $y = 0.80x^{0.75}$ ±2 S D of the control group

highly significant ($r=0.98$). This high degree of correlation can be taken as an indication that 1) A constant electrolytic balance was maintained throughout the test or in the broad sense factors acting on the concentration mechanism were constant in all the tests 2) There was no need to maintain a prior period of hydropenia

Studies of Calcagno show that when the patient is a newborn or infant the values of C_{osm} and T_{H_2O} should be referred to 100 ml GFR in order to be comparable to those of the older child and adult (3) With the method of study used by us this correction to GFR was only necessary in the first months of life (Table 3) In children over six months the T_{H_2O} related to 1.73 m² b s did not show—at any level of C_{osm}—significant differences from children over a year old This is of interest since in patients over six months old the results of osmolar clearance can well be related to body surface The curve obtained in these children related to 1.73 m² b s ($y=0.88x^{0.73}$) is quite similar to the one referred to 100 ml GFR ($y=0.80x^{0.73}$)

In the PDI patients the hypertonic mannitol induced the formation of negative or subnormal values of T_{H_2O} (Fig 2A) In the first part of the test (open signs) the response was similar when there was a deficient secretion of ADH (PDI) or if the collecting tubule was insensitive to it (NDI) (Fig 2B) Differences of degree regarding the lack of ADH should be interpreted proportionally to the lesser or greater excretion of C_{H_2O} in the presence of the osmotic overload The PDI patient able to get subnormal amounts of T_{H_2O} under hypertonic mannitol infusion without vasopressin would enter into the group that Miller has called partial diabetes insipidus (14) The different behavior of the cases studied in relationship with the degree of endogenous ADH deficit will help in prescribing the therapeutic dosage of exogenous hormone In the second part of the test when exogenous vasopressin was infused (closed signs) a differential diagnosis was established between pituitary and nephrogenic diabetes insipidus (Fig 2) When there was a lack of ADH the C_{H_2O} should diminish making positive the free water reabsorption (T_{H_2O}) The maximum volumes of C_{H_2O} and the total lack of response to the exogenous vasopressin are observed in patients with NDI

The antidiuretic action of carbamazepine was evaluated in four patients with PDI through the method of osmolar clearance presented In three patients the drug proved effective showing a fairly significant reduction in the C_{H_2O} during the first part of the test—at similar levels of C_{osm}—(Table 4) In J B R the T_{H_2O} became positive without the exogenous addition of vasopressin In agreement with the experience of other authors the antidiuretic effect of carbamazepine was greater in secondary PDI than in the idiopathic forms This fact shows a minimal need of ADH to condition the effectiveness of the drug (18) Hence in R B R we attribute the lack of effect to the total absence of ADH showing C_{H_2O} values similar to those of the NDI patients (Fig 2)

In the two patients with idiopathic PDI whose response to carbamazepine was negative (R B R) or weak (A B C) we tested the effect of clofibrate alone and in association with carbamazepine The results of Table 5 show how in R B R none of the drugs or combination of them took effect on the formation of free water In patient A B C —familiar idiopathic PDI—there was a difference between the effect achieved when the two drugs were administered separately or together From the results of Table 5 and Fig 3 we can deduce that the presence of carbamazepine was necessary so that the clofibrate would have an additional antidiuretic action over what was achieved with the former

The observation allows us to postulate the different action mechanism of both drugs with the action of carbamazepine being permissive so that the clofibrate can act Taking into consideration the experiences that localize the

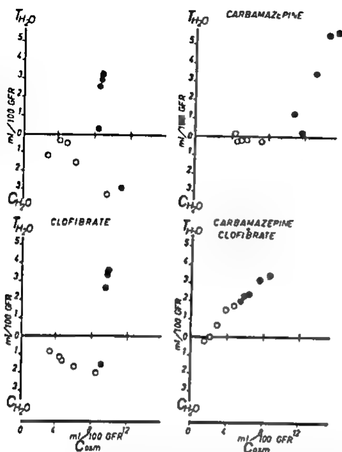


Fig 3 Carbamazepine and clofibrate effect on patient A B C with pituitary diabetes insipidus Values obtained under mannitol infusion before (open symbols) and after (closed symbols) vasopressin addition

water reabsorption The way of action of these hypertonic solutions is established at the endocrine level stimulating ADH secretion and at the renal level inducing a greater delivery of chloride and sodium to those tubular sites that are responsible for the countercurrent mechanism (22)

Studies in humans show a progressive increase in T_{H_2O} during diuresis induced by hypertonic mannitol (11) This means that besides the ADH permeabilizing action on the collecting tubule, the reabsorption of chloride and sodium at the diluting segment of Henle s loop is increased through a greater delivery from proximal segments (11, 22)

Initial studies with hypertonic mannitol showed the existence of a maximum tubular limit in the formation of T_{H_2O} (1 20 23) Goldberg et al reject this concept on comparing the effects of hypertonic overloads of saline and mannitol They show how an infusion of hypertonic saline progressively increases the T_{H_2O} in relation with the C_{osm} without any evidence of a saturation capacity of the transport in the diluting segment of Henle s loop (11) The T_{H_2O} maximum tubular limit observed with the mannitol load is attributed on one hand to the decrease of sodium and chloride concentration within the tubules and on the other hand to the greater blood flow in vasa recta both induced by the mannitol osmotic diuresis (11 21)

In order to avoid the mannitol induced ionic depletion sodium chloride and potassium were supplemented to the mannitol infusion in amounts to maintain serum levels constant In this way the limiting effect in the reabsorption of free water was eliminated at the C_{osm} levels used in the study The hypertonic sodium overload which is not well tolerated by infants is also avoided On the other hand the hypertonic mannitol produced enough increase of plasmatic osmolality to maximally stimulate the endogenous secretion of ADH The vasopressin given exogenously during the second part of the test would induce a maximum antidiuretic effect if endogenous ADH should be insufficiently produced

At low levels of C_{osm} the values of T_{H_2O} obtained by us were similar to those that other authors refer to by using hypertonic mannitol without the addition of sodium and potassium and with a previous period of hydropenia (1 11 20 23) Nevertheless all of these indicate a limit in the formation of T_{H_2O} which in the report of Sommerschild reaches at a C_{osm} of 8–12 ml/min/1 73 m² b s (20) In the cases studied by us and according to Goldberg the increase of T_{H_2O} occurred in relationship with the increase of C_{osm} according to a geometric curve without any maximum limit being observed at least up to C_{osm} of 20–22 ml/100 ml GFR

In the control group the statistical correlation between T_{H_2O} and C_{osm} shows itself to be

ighly significant ($r=0.98$). This high degree of correlation can be taken as an indication that 1) A constant electrolytic balance was maintained throughout the test or in the broad sense factors acting on the concentration mechanism were constant in all the tests. 2) There was no need to maintain a prior period of hydropenia.

Studies of Calcagno show that when the patient is a newborn or infant the values of C_{osm} and T_{H_2O} should be referred to 100 ml GFR in order to be comparable to those of the older child and adult (3). With the method of study used by us this correction to GFR was only necessary in the first months of life (Table 3). In children over six months the T_{H_2O} related to 1.73 m² b.s. did not show—at any level of C_{osm}—significant differences from children over a year old. This is of interest since in patients over six months old the results of osmolar clearance can well be related to body surface. The curve obtained in these children related to 1.73 m² b.s. ($y=0.88x^{0.75}$) is quite similar to the one referred to 100 ml GFR ($y=0.80x^{0.75}$).

In the PDI patients the hypertonic mannitol induced the formation of negative or subnormal values of T_{H_2O} (Fig. 2A). In the first part of the test (open signs) the response was similar when there was a deficient secretion of ADH (PDI) or if the collecting tubule was insensitive to it (NDI) (Fig. 2B). Differences of degree regarding the lack of ADH should be interpreted proportionally to the lesser or greater excretion of C_{H_2O} in the presence of the osmotic overload. The PDI patient able to get subnormal amounts of T_{H_2O} under hypertonic mannitol infusion without vasopressin would enter into the group that Miller has called partial diabetes insipidus (14). The different behavior of the cases studied in relationship with the degree of endogenous ADH deficit will help in prescribing the therapeutic dosage of exogenous hormone. In the second part of the test when exogenous vasopressin was infused (closed signs) a differential diagnosis was established between pituitary and nephrogenic diabetes insipidus (Fig. 2). When there was a lack of ADH the C_{H_2O} should diminish making positive the free water reabsorption (T_{H_2O}). The maximum volumes of C_{H_2O} and the total lack of response to the exogenous vasopressin are observed in patients with NDI.

The antidiuretic action of carbamazepine was evaluated in four patients with PDI through the method of osmolar clearance presented. In three patients the drug proved effective showing a fairly significant reduction in the C_{H_2O} during the first part of the test—at similar levels of C_{osm}—(Table 4). In J. B. R. the T_{H_2O} became positive without the exogenous addition of vasopressin. In agreement with the experience of other authors the antidiuretic effect of carbamazepine was greater in secondary PDI than in the idiopathic forms. This fact shows a minimal need of ADH to condition the effectiveness of the drug (18). Hence in R. B. R. we attribute the lack of effect to the total absence of ADH showing C_{H_2O} values similar to those of the NDI patients (Fig. 2).

In the two patients with idiopathic PDI whose response to carbamazepine was negative (R. B. R.) or weak (A. B. C.) we tested the effect of clofibrate alone and in association with carbamazepine. The results of Table 5 show how in R. B. R. none of the drugs or combination of them took effect on the formation of free water. In patient A. B. C.—familiar idiopathic PDI—there was a difference between the effect achieved when the two drugs were administered separately or together. From the results of Table 5 and Fig. 3 we can deduce that the presence of carbamazepine was necessary so that the clofibrate would have an additional antidiuretic action over what was achieved with the former.

The observation allows us to postulate the different action mechanism of both drugs with the action of carbamazepine being permissive so that the clofibrate can act. Taking into consideration the experiences that localize the

central action of the carbamazepine stimulating the liberation of ADH (9 13) and the experiences of Moses, rejecting the action of clofibrate in the collecting tubule (15) we could postulate a potentiating action of the latter drug on the circulating ADH (7) If as Moses affirms both drugs have a central action the result observed in the patients studied here would advocate a permissive action at different level of carbamazepine on clofibrate

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ABSTRACT Nielsen H E Lundh S Jacobsen S V and Høiby N (Paediatric Department TG Rigshospitalet Proteinlaboratoriet and Statens Seruminstitut Department of Clinical Microbiology at Hvidovre Hospital Copenhagen Denmark) Hypergammaglobulinemic purpura in cystic fibrosis Acta Paediatr Scand 67 443 1978.—Four patients are presented aged 14 to 20 years with cystic fibrosis and recurrent purpura of the legs They have polyclonal increase of Ig but no intermediate complexes demonstrable by ultracentrifugation The 4 patients differ from other patients with cystic fibrosis by a rapid deterioration of the clinical condition after the establishment of permanent pulmonary infection and also by their proneness to Haemophilus influenzae infections The clinical and laboratory findings are compatible with the diagnosis of Waldenstrom s hypergammaglobulinemic purpura The heterogeneity of this syndrome is discussed

KEY WORDS Purpura hyperglobulinemic cystic fibrosis *Haemophilus influenzae*

Waldenstrom (17–19) described the syndrome of hypergammaglobulinemic purpura (HP) consisting of recurrent purpura of the legs together with increased immunoglobulin concentration in the serum Additional features are elevated erythrocyte sedimentation rate slight anemia and a normal coagulation status Young women are most frequently affected and only a few cases have been described in children (10 12 18 20 21) The pathogenesis of the syndrome is unknown

The syndrome occurs both in a primary form without evidence of other diseases and also in a secondary form combined with other diseases notably Sjogren s syndrome systemic lupus erythematosus and sarcoidosis The appearance of the latter diseases may be delayed for many years after the appearance of the purpura

Intermediate complexes with sedimentation rates between 7 S and 19 S have been demonstrated by ultracentrifugation in the serum of a majority of adult patients with the primary (2 3 13 15) as well as the secondary form of HP (13) and also in the serum of one of three children (10 21)

In 4 children with cystic fibrosis (CF) and hypergammaglobulinemia following chronic pulmonary infection we observed purpura closely resembling that described by Waldenström when the children were approaching young adulthood The simultaneous occurrence of purpura and CF has not been described previously Analytical ultracentrifugation could not demonstrate intermediate complexes in the sera of the 3 patients examined by this technique The pathogenesis of the purpura and its possible relation to Waldenstrom s hypergammaglobulinemic purpura is discussed

FINDINGS

The 4 cases described below were found among 156 CF patients controlled usually once per month over an average period of 5 years The 4 patients all had the typical clinical features of CF and an abnormal sweat test Common to them was that their age was relatively high and that the CF had progressed extensively with severe chronic pulmonary infection at the time the purpura appeared —in fact purpura would seem to be a terminal phenomenon (Table 1)

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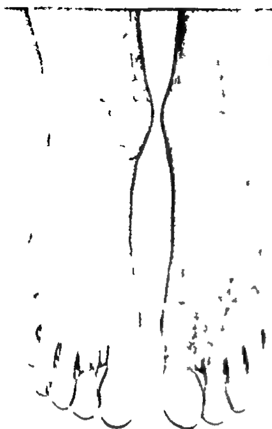


Fig 1 Typical purpura of the legs in patient no 109

linemic purpura (17–19) The purpura was chronically remitting the petechiae were confined to the legs and were most pronounced distally (Fig 1) Two patients (nos 84 and 144) noticed aggravation of the purpura after exposure to cold The appearance of purpura was in some cases accompanied by edema itching and burning and the petechiae left a brown pigmentation on disappearance There was no bleeding tendency abdominal pain arthritis splenomegaly or lymph node enlargement

Two of the patients showed peculiar features One of them (no 24) had purpura only for a few days and had no recurrence before he died 6 months later He was treated with plasmapheresis twice during this period this may have changed the spontaneous course The other patient (no 109) had urticaria in addition in the purpura The activity of the purpura and of the urticaria was independent in time and while the urticaria responded well to antihistaminics the purpura did not

The laboratory findings (Table 2) were also compatible with those in hypergammaglobulinemic purpura There was a polyclonal increase of Ig also found in the control patients (all our other CF patients aged 14 years and over) IgG was elevated in all the purpura patients and IgA was also elevated in 3 of them while IgM was normal in all cases The serum concentration of IgD and IgE was measured in 2 patients and was found to be normal The erythrocyte sedimentation rate was elevated in all cases We found no serum components with sedimentation rates between 7 and 19 S in any of the 3 patients examined using an analytical ultracentrifugation technique identical to that of Capra et al (2) (Fig 2)

There was no anemia by ordinary standards but because of the chronic lung disease the hemoglobin would be expected to be well above the lower normal limit The coagulation status was normal except for a slight prolongation of prothrombin time which is often found in CF patients with advanced disease

Skin biopsy was done in 2 patients In one of them Ig was deposited in the vessel wall in the other there were no signs of vasculitis The post mortem examinations showed no special features compared with other CF patients

The 4 patients had consistent growth of *pathogenic microorganisms in the sputum* in spite of repeated specific antibiotic treatment They were all at some time infected with *Pseudomonas aer* (Fig 3*a*) but Pseudo-

Table 1 *Clinical features of 4 CF patients with purpura*

Case number	109	144	24	84
Sex	♀	♂	♂	♂
Fibroblast type	n t [b]	I	I	I
Age at diagnosis (years)	12 2/12	9 9/12	6/12	1
Age at appearance of purpura (years)	20 2/12	14 10/12	14 1/12	17 8/12
Duration of the purpura (years)	1 6/12	5/12	8 days	1 (still alive)
Interval from appearance of purpura to death (years)	1 6/12	5/12	6/12	still alive
Age at death (years)	21 8/12	15 3/12	14 7/12	still alive

According to Danes et al (4)
[b] Not tested

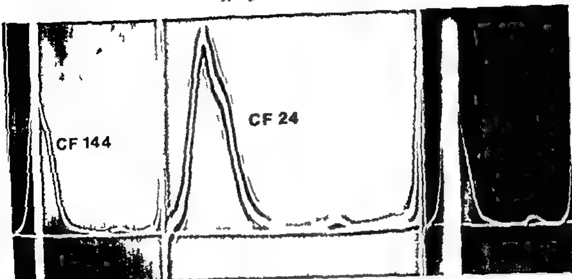


Fig 2 Analytical ultracentrifugation of the sera from 3 patients with CF and purpura The sera were drawn at the first appearance of purpura The Schlieren patterns were taken 45 minutes after the maximum speed of 52640 rpm was reached There is no sedimenting material in the region between 7S and 19S except in serum no 144 in which 2% of the protein sediments in the region Serum no 144 had been frozen for a year prior to analysis while the other sera were fresh and the 2% may be an artefact due to storage (5)

monas disappeared in 2 of them although they were in the terminal stage of the disease—a very uncommon finding There was a strikingly high frequency of Haemophilus infections (Fig 3*b*) in fact all 4 purpura patients were chronically infected with *Haemophilus influenzae* for some time prior to the appearance of purpura In this respect they clearly differed from the control group while they did not differ with respect to the frequency of infections with other bacteria—almost exclusively *Staph aureus* and *Pneumococci*—or the number of antibody specificities against *Haemophilus* (Fig 3*c*) and *Pseudomonas* (data not shown) The clinical deterioration was unusually rapid in the 3 patients who died In our clinic it is generally the case that CF patients are chronically infected with *Pseudomonas* for 3 years prior to death (9)

DISCUSSION

The type of purpura seen in our patients closely resembles hypergammaglobulinemic purpura (HP) as described by Waldenstrom (17–19) although our patients show some special

Table 2 *Laboratory findings in 4 CF patients with purpura*

Analysis	Case number 109	144	24	84
Plasma IgA (0 56–3 30 g/l)	4 22	4 79	1 19	3 36–4 32[a]
Plasma IgG (6 8–15 7 g/l)	25 2	26 7	35 1	17 6–21 7
Plasma IgM (0 18–1 79 g/l)	0 94	0 39	0 47	0 66–0 95
Hemoglobin (♂7 9–10 5 mmol/l) (♀7 0–9 4 mmol/l)	7 3–7 9	7 6–8 7	6 8	8 9–9 5
Sedimentation rate (<10 mm/h)	56–58	11–34	40	20–26
Intermediate complexes in serum (cf Fig 3)	n t	0[d]	0	0
Rheumatoid factor in serum	0	n t	n t	0
Cryoglobulins in serum	n t	n t	0	0

Normal range
Smallest and largest value from the period in which petechiae appeared
Not tested
Not present

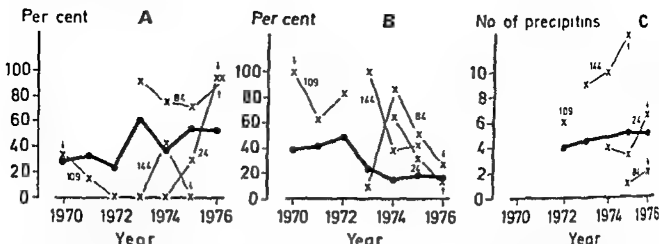


Fig 3 (A) Frequency of sputum cultures in which *Ps aeruginosa* was seen and cultured in individual patients with purpura (×—× patient no indicated) and in controls (●—●) The controls are all our other CF patients aged 14 years and over Each point indicates the mean value for 13–29 control patients Arrows indicate year in which purpura started The trend of the control curve may be an effect of an increase in the survival time after the establishment of Pseudomonas infection (B) Frequency of sputum cultures in which *Haemophilus influenzae* was seen and cultured The trend of the control curve is probably caused by the introduction of ampicillin (C) Number of antibody specificities directed against *Haemophilus influenzae* in serum determined by crossed immunoelectrophoresis (7 8)

features by comparison with the common findings in HP they are younger and have chronic pulmonary infection as an explanation for their hypergammaglobulinemia While HP may occur in conjunction with a number of other diseases notably the so called collagen diseases its coexistence with CF has not been described previously

The possibility exists that the purpura in our patients may be a side effect of ampicillin with which they were all treated repeatedly This drug has in rare instances caused purpura (1 6) We consider this unlikely here because only one of them was treated with the drug within the last month before the first appearance of purpura Furthermore they all had courses of treatment with ampicillin after the first bout of petechiae without any discernible effect on the purpura

Recently it has been shown that most but not all HP patients have rather high concentrations of intermediate complexes in their serum sedimenting between the 7 S and 19 S peaks (2 3 10 13 15) and also that these may be gammaglobulin antigammaglobulin complexes (2) Absence of intermediate complexes as in our patients does not rule out the possibility that circulating immune complexes may be present for judged by a complement-consumption technique they seem to be present in many chronically infected CF patients (14) and there is some experimental evidence that immune complexes may provoke pulmonary damage (11 22)

The pathogenesis of HP is unknown It has been suggested (2) that the intermediate complexes may provoke infarction and extravasation of blood in the small vessels This of course does not explain the purpura in cases without intermediate complexes In our cases there was a striking coincidence of chronic Haemophilus infection and purpura One might speculate that an abnormal immune response against Haemophilus would result in persistent pulmonary infection as well as purpura but we have no evidence to support that in fact the quantitative immune response against Haemophilus as measured by crossed immunoelectrophoresis (8 9) in the 4 purpura cases was comparable to that of the control group

The pathogenesis of the syndrome is still unknown and it may well be entirely different in different cases Our findings strengthen the impression from the literature (2 15 16) that the syndrome of HP is heterogeneous with re

gard to both pathogenesis and laboratory findings emphasizing that it should not be regarded as [redacted] diagnostic entity without [redacted] more precise definition

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FREQUENCY OF PSYCHOTROPIC DRUG PRESCRIBING FOR CHILDREN IN TAMPERE FINLAND

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ABSTRACT Kreula E and Hemminki [redacted] (Department of Public Health Sciences University of Tampere Finland) Frequency of psychotropic drug prescribing for children in Tampere Finland Acta Paediatr Scand 67 449 1978.—The purpose of the present study was to determine the frequency with which psychotropic drugs are prescribed for children under 10 years of age in outpatient care in Finland This frequency was estimated from the reimbursements paid by the Social Insurance Office of Tampere City in 1974 Every third psychotropic drug prescribed for children born in 1965–74 was included This result[redacted] in 319 children with 375 psychotropic drug prescriptions About 4% of children under [redacted] years old [redacted] the Tampere area had received within one year s time one or more psychotropic drugs in outpatient care About half of the psychotropic drugs were prescribed by general practitioners Sedatives were the most frequently prescribed drugs especially in the younger age groups Antidepressants and antiepileptics were common in the older age groups The most commonly specified indications were fever and restlessness

KEY WORDS Psychotropic drugs children outpatient survey

The abundant prescribing of psychotropic drugs has often been discussed The use of these drugs in outpatient care has aroused particular interest (see e g Hemminki (6)) though research has been concentrated mainly on problems concerning adults Data on psychotropic drug consumption among children are scant and even the frequency with which psychotropic drugs are prescribed for this population is not known

Determining the efficacy and adverse effects of drugs [redacted] more difficult among children and the indications for psychotropic drugs in this group are not well established These problems have been discussed in particular in the United States and the focus has been on enuresis and hyperactivity (2 3 4 9 10)

The purpose of the present study was to establish the frequency with which psychotropic drugs are prescribed in outpatient care in Finland The city of Tampere in southern Finland was chosen for the survey area With its 165978 inhabitants in 1974 Tampere ranks as the second largest city in Finland

MATERIAL AND METHODS

The frequency of psychotropic drug prescriptions was estimated from the reimbursements paid by the Social Insurance Office of Tampere City Apart from Tampere itself the social insurance district consists of the neighbouring rural communes Pirkkala and Ylojarvi

In 1974 the population 0–9 years old was as follows Tampere 2[illegible]81 (10898 girls and 11383 boys) Pirkkala 142[illegible] (66[illegible]+760) and Ylojarvi 1693 (852+841) (7) All prescribed psychotropic drugs are eligible for reimbursement [redacted] the person has her social security card with her reimbursement is done in pharmacies [redacted] the connection of the purchase Otherwise the person can get the reimbursement afterwards In general very few persons do not use their right to reimbursement When a person buys drugs outside Tampere the drugs are not reimbursed by the Tampere Social Insurance Office Purchases made in Tampere by citizens of other social insurance districts are reimbursed by the Tampere Social Insurance Office

Every third psychotropic drug ordered for children born in 1965–74 and filed in 1974 was examined The next non psychotropic drug prescription in the file for children in this age group was selected as a control When the sample included several psychotropic drugs for the same

Table 1 *Distribution of psychotropic drug presciptions by sex*

	N	%	Rate per 100 children in the Tampere area
Boys	190	60	4 4
Girls	129	40	3 1
Total	319	100	3 8

child all these drugs were recorded for this child The controls for the second and third drugs have been excluded All information available in the prescriptions was collected including the name of the drug dose indication and physician s name The duration of therapy was calculated by dividing the total number of tablets (ml) by the daily dosage The physician s specialty was obtained from the catalogue of doctors practising in Tampere

319 children with 375 psychotropic drug prescriptions and 317 children with control drug prescriptions form the sample If not mentioned otherwise only the information of the first psychotropic drug is considered in Results The second and third psychotropic drugs usually belonged to the same drug group as the first In four cases (in two psychotropic drug prescriptions and two controls) the age of the child was not known and these were excluded from Table 2 Three psychotropic drugs belonging to other groups than those listed below were excluded from Tables 2 3 and 5

The classification of psychotropic drugs was based on *Remedia Fennica* (5) and following drugs were included The terms used in the tables and text are in italics and the symbols in parentheses refer to *Remedia Fennica*

1 *Sedatives* hypnotics (11a)
2 *Anxiolytic* drugs (minor tranquillizers) (11b)
3 *Antipsychotic* drugs (11c)
4 Psychostimulants *antidepressants* (11d)
5 *Drugs* for *migraine* (11g)
6 *Antiepileptics* anticonvulsants (11h)
7 *Hidden psychotropic drugs* Preparations which include psychotropic drugs in addition to other active incredients and which are not classified as psychotropic drugs

The ages of the children in the sample were calculated from the year of the birth When estimating the prescription frequency the number of children reimbursed in each age group was multiplied by three and divided by the number of the children of that age in the Tampere area

RESULTS

Fifty one percent of the children under 10 years were boys and 49% were girls in the Social Insurance District of Tampere (7) Sixty percent of those who had received psychotropic drugs and 57% of the controls were boys (Table 1) About 4% of children had received one or more psychotropic drugs in outpatient care within one year The frequencies of psychotropic drug prescribing in different age groups are given in Table 2 Children born in 1972 (about 2 years old) and in 1966 (about 8 years old) had received psychotropic drugs most frequently The distribution of control drug prescriptions was not similar Compared with psychotropic drugs these drugs were prescribed more often for children aged 0 to 5

Table 2 *Percentage distribution of different psychotropic drugs by age*

Age (years)	Number of children	Rate per 100 children in the Tampere area	Sedatives	Anxiolytic drugs	Antipsychotics	Antidepressants	Migraine drugs	Antiepileptics	Hidden psychotropic drugs	Total
0	1	(0 0)	–	–	(100)	–	–	–	–	(100)
1	25	3 3	44	24	4	–	–	4	24	100
2	44	5 6	50	16	–	–	11	–	23	100
3	20	2 7	55	20	–	5	–	10	10	100
4	24	3 0	54	8	–	8	4	13	13	100
5	33	4 0	37	15	–	15	15	12	6	100
6	41	4 6	14	24	5	20	10	7	20	100
7	35	3 7	17	6	–	32	14	17	14	100
8	59	6 4	12	17	3	14	8	31	15	100
9	32	3 6	9	9	3	22	16	28	13	100
Total	314	3 7	29	16	2	13	9	15	16	100

Table 3 *Percentage distribution of psychotropic drug prescriptions by the mean duration of the therapy*

Duration of therapy days	1st drug N=316	2nd drug N=43	3rd drug N=13
<10	46	78	15
10–30	16	9	8
>30	35	[illegible]	77
Total	100	100	100

Table 2 also shows the distribution of various psychotropic drugs by age As described in Methods only the first psychotropic drug was included Sedatives were the most frequently prescribed drugs especially in the younger age groups Antidepressants and antiepileptics were common in the older age groups About 60% of the psychotropic drugs belonged to the first four groups (sedatives anxiolytics antipsychotics and antidepressants) Of the second drugs about half were antiepileptics The third drug belonged to this group in about 70% of cases

The mean duration of therapy for the first psychotropic drug prescription was 41 days [illegible] days for the second and 98 days for the third psychotropic drug prescription (20% of the first psychotropic drugs and 10% of the second and third drugs were ordered to be taken only when needed For these drugs the duration of therapy was counted as if they were ordered to be taken regularly using the recommended dosage) In half of the first psychotropic drug prescriptions the duration of therapy was under 10 days but in the case of the second and third drug the duration of therapy was longer (Table 3) Antiepileptics had the longest duration of therapy (mean 127 days) The mean duration of therapy was 76 days for antidepressants 46 days for antipsychotics 41 days for anxiolytic drugs and 7 days for sedatives

Table 4 *Percentage distribution of drug prescriptions by the prescribing physician s specialty*

Specialty	Psychotropic drug (N=314)	Control drug (N=317)
General practitioner	54	74
Pediatrics	27	13
Psychiatry or child psychiatry	6	–
Otolaryngology	[illegible]	[illegible]
Dermatology	6	3
Other specialities	1	[illegible]
	100	100

Table 4 shows the drug prescribing by physicians specialty Fifty four percent of the psychotropic drugs were prescribed by general practitioners If the specialties of the prescribers of the control drugs are used as estimations of the frequencies of different physician contacts we may conclude that reckoned per consultation pediatricians prescribed

Table 5 *Percentage distribution of indications by drug group*

Drug group	No of prescriptions	Indications								No Indication	Total
		Fever	Restless ness	Nausea	Enuresis	Allergic symptoms	Cough dyspnea	Head ache	Epilepsia		
Sedatives	91	5[illegible]	16	–	–	–	–	–	2	30	100
Anxiolytic drugs	49	–	8	–	–	35	–	–	4	53	100
Antipsychotic drugs	7	–	14	–	–	–	–	–	–	86	100
Antidepressants	[illegible]	[illegible]	5	–	17	–	–	–	–	76	100
Migraine drugs	30	–	–	53	–	–	–	23	–	74	100
Antiepileptics	46	–	–	–	–	–	–	–	17	83	100
Hidden psychotropic drugs	49	10	6	–	[illegible]	–	14	–	12	56	100
	314	17	8	6	5	5	2	2	[illegible]	51	100

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6	41	4 6	14	24	5	20	10	7	20	100
7	35	3 7	17	6	–	32	14	17	14	100
8	59	6 4	12	17	3	14	8	31	15	100
9	32	3 6	9	9	3	22	10	28	13	100
Total	314	3 7	29	16	2	13	9	15	16	100

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GROWTH BODY WEIGHT AND INSULIN REQUIREMENT IN DIABETIC CHILDREN

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ABSTRACT Petersen H D Korsgaard Bente Deckert T and Nielsen E (Steno Memorial Hospital Gentofte Denmark) Growth body weight and insulin requirement in diabetic children Acta Paediatr Scand 67 453 1978.—The weight and height development of 51 boys and 48 girls who had developed diabetes prior to the age of 15 years was followed for a minimum of 3 years mean 8.3 years up to the age of 18 for the girls and 20 for the boys In addition the insulin requirements were recorded Weight and height proved to be within the normal range and height at onset of diabetes was normal After a long duration of diabetes however there occurred a reduction of height increment of about 1/2 cm/year This reduction was greater in children who rarely attended as compared with those who frequently attended a sub-specialized clinic The daily dose of insulin increased with age the greatest increase coinciding with the growth spurt During the first 4 years of diabetes the 24-hour dose/kg body weight increased indicating a decreasing endogenous insulin production Later it was constant at around 0 9 i u /kg Modern management of diabetic children leads to normal adult stature

KEY WORDS Diabetes mellitus children growth body weight insulin requirement

Insulin is one of the most anabolic hormones Children with decompensated insulin deficiency diabetes therefore show retardation of growth despite an elevated plasma level of growth hormone Deckert et al (5) found in 16% of 119 children with diabetes before the age of 15 diagnosed between 1922 and 1932 a final adult body height more than two standard deviations below the mean In a similar Swedish series Larsson et al (20) reported in 22% of diabetic children a final height more than two standard deviations below the mean height attained by non diabetic children In a British series (25) 13% of the children had a final height of more than three standard deviations below the mean height of non diabetic children Retardation of growth may be accompanied by obesity hypogonadism and hepatomegaly (the Mauriac syndrome) (23) To-day the Mauriac syndrome is rare (11) but it is doubtful whether subcutaneous insulin therapy combined with diabetic diet and exercise can re-establish such a favourable metabolic state in diabetic children that a normal height increment can take place We therefore studied the height and weight development of diabetic children and tried to ascertain whether close contact with a sub-specialized clinic affected the growth of such children Furthermore the patients insulin requirements were recorded

MATERIALS AND METHODS

The material comprises 99 children 51 boys and 48 girls who were attending the Steno Memorial Hospital The children were admitted to the study consecutively according to their date of birth All had developed insulin dependent diabetes prior to the age of 15 and the duration of the disease was at least 3 years The age at onset of diabetes is apparent from Fig 1 which shows that in about 50% diabetes had been diagnosed before school age

Fig 2 illustrates the composition of the material according to the duration of diabetes About half of the children were seen for the first time within 5 months of onset All had been followed for at least 3 years in the Steno Memorial Hospital and the follow up of height increment was concluded after a total duration of 831 years at the latest at 18 years of age for the girls and at 20 for the boys The diabetes therapy was checked at a total of 6668 out patient visits distributed on 80 patients (10 visits/year/

psychotropic drugs more frequently than other physicians

Indication was given only in 49% of the psychotropic drug prescriptions (Table 5) The most common indications were fever and restlessness In each age group the majority of indications could be classified as somatic or psychosomatic diseases fever nausea allergic symptoms dyspnea and headache Prescriptions without indications were most common among the children over 6 year old Fewest indications were given for antipsychotics, antidepressants and antiepileptics For instance in only 17% of antiepileptic drugs were convulsions recorded as an indication Indications for migraine drugs had been recorded most often Most of the sedatives were given for fever and for restlessness Various allergic symptoms were the main indications for anxiolytic drugs Psychostimulants were often prescribed for enuresis Various hidden psychotropic drugs were frequently prescribed for cough and dyspnea

DISCUSSION

Psychotropic drugs were prescribed for about 4% of children under 10 years of age during the year 1974 More than half of the specified indications were of a somatic or psychosomatic nature (e g fever and nausea) Bain (1) has reported similar findings from a Scottish general practice of five doctors In his material, 6% of children under 12 years of age received one or more prescriptions for psychotropic drugs during 1971 and 13% of all psychotropic drug prescriptions issued during that year were for children Most common indications in the Scottish study were behavioural disorders and enuresis

Our figure for the frequency of psychotropic drug prescriptions may be an underestimate since it is possible that reimbursements were not always sought The purchases outside the area and those made in the Tampere area for children from other communes may bring some further bias the direction of which is not known But in the case of children buying drugs outside the home community is considered to be infrequent in Tampere (8) Nevertheless our survey indicates that the prescribing of psychotropic drugs for children is not rare This study does not consider the issues of the proportion of these drugs that were really indicated nor what were the benefits and side effects of these drugs Since a child s brain is in the developing stage both in a somatic and a psychological sense a future evaluation of these issues would seem both prudent and important in the total assessment of psychotropic drug prescribing for children

ACKNOWLEDGEMENTS

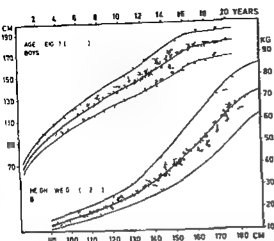
We wish to thank the Social Insurance Institution of Finland for their allowing us to use the drug prescription data We also thank the Tampere Social Insurance Office for help and advice in the course of the study

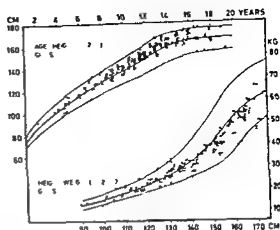
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Figs 3 and 4 All height and body weight measurements ( ) in 51 boys and 48 girls with diabetes followed at the Steno Memorial Hospital The curves represent the mean ±2 S D of non-diabetic children according to Broman et al (3) and Karlberg & Perman (16)

majority exhibited a greater than normal height increment during the subsequent 3 years (Fig 6)

Body weight seemed to be unaffected by the duration of diabetes It was constant around 100% being a little lower only during the first year of diabetes

The 24 hour dose of insulin increased with age (Fig 7) The increase was most pronounced during puberty and therefore earlier in girls than in boys (not shown) Girls had a somewhat higher daily dose of insulin/kg and 24-hour dose/square metre body surface than boys from the age of 10 but it has not been elucidated whether this difference is independent of puberty Plotting the 24 hour dose of insulin/kg against the duration of diabetes shows that the dose was constant after a duration of 4 years In the course of the first 4 years the daily dose/kg increased by about 50% (Fig 8)

DISCUSSION

According to the present finding the height and weight of diabetic children are within the ranges of normal At the onset of diabetes the body height in the present series was about 2% above mean height which is not a significant difference from non diabetics Some authors have reported that children with newly diagnosed diabetes are taller than other children of the same age (6 25 28) Others have been unable to confirm this (4 9 11 13 19 27) The explanation that some authors have found children at the onset of diabetes to be taller than non diabetic children may be due to difficulties in procuring adequate control series In Tattersall and Pyke s series of monozygotic twins in which one of each pair developed juvenile diabetes there was no difference in height between the diabetic and the non-diabetic twin at onset of diabetes Thus although height appears to be normal at the onset of diabetes in children retardation of

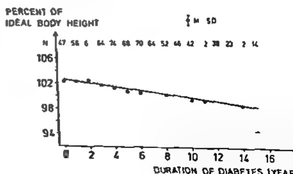


Fig 5 Per cent of ideal body height in 99 diabetic children in relation to duration of diabetes

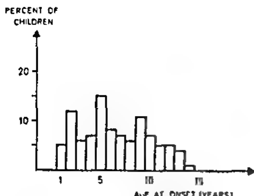


Fig 1 Age at onset of diabetes in 99 children

patient) and during 511 brief admissions to the Hospital distributed on 98 patients (0 63 admission/patient/year) All the children had a diet with 2–3 g protein/kg body weight/24 hours a fat content of about 40% of the total calories (about 1 000 calories+100 calories/year of age) and a carbohydrate content of about 40–45% of the total calories 90% of the children received insulin twice daily during the treatment period beginning with one injection daily followed by two injections daily after 3–5 years of treatment

Height and weight were measured on each admission At the out patient visits the weight was recorded (lightly dressed without shoes) and the height at the first visit in each calendar year The measurements of weight and height were performed by trained nurses using the same weight and the same height measure A total of 718 height measurements and 773 weighings were carried out Between two height measurements there was a minimum interval of 12 months The deviation from the mean height of non diabetic girls and boys was recorded as the % of ideal height the children s real height being related to the mean height reported by Broman et al (3) and Karlberg & Perman (16) for girls and boys of different age groups Body weight was expressed as % in relation to the ideal weight for the height as reported by Broman et al (3) and Karlberg & Perman (16) This control material was chosen because the newest Danish control material (1) from 1971–1972 only included figures for 11 000 school children aged 7–18 Over the age of 10 the control figures for mean body height were 1–2 cm lower than mean body height in the newest Danish control material and over the body height of 160 cm the body weight in the control material was 1–2 kg higher than in the newest Danish material Below the age of 10 the body height and below the height of 160 cm the body weight were identical in the two groups Surface area was calculated by the Du Bois & Du Bois formula (7) The 24 hour dose of insulin was recorded only for healthy ambulatory in patients during the 24 hours before their discharge

RESULTS

Figs 3 and 4 list all the height and weight measurements It is evident that to day the height and weight of diabetic children is within the normal range Out of 718 height measurements only 1 5% were more than two standard deviations below the mean height and 1 5% two standard deviations above the mean height of boys and girls in the various age groups

1 4% of all the weight measurements exceeded two standard deviations of the ideal weight and 0 8% were more than two standard deviations below the ideal weight Mauriac s syndrome was not observed

Investigation of the deviations from ideal height in relation to the duration of their diabetes showed a significant reduction in height increment amounting to 3 4% of the ideal height after a duration of 12 years (Fig 5) Patients in whom the diabetes had set in before the age of 8 and who had from the onset and for more than 10 years been treated regularly in the Steno Memorial Hospital (19 patients) also showed a significant retardation of growth ($p<0\ 02$) but considerably less than a group of diabetic children whose diabetes had also set in before the age of 8 and which was of more than 10 years duration but who had been followed for less than 50% of the duration in the Steno Memorial Hospital (1 3% as against 5 6% of the ideal height in the course of duration of 12 years) In patients whose treatment at the Steno Memorial Hospital was started more than 2 years after the onset the

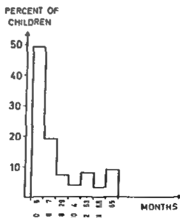


Fig 2 Duration of diabetes in 99 children when first seen at the Steno Memorial Hospital

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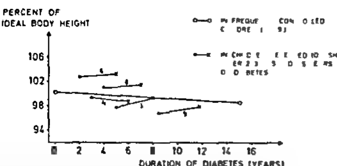


Fig 6 Development of body height during 3 years in children referred to the Steno Memorial Hospital (NSH) after 2 3 4 5 and more than 5 years of diabetes

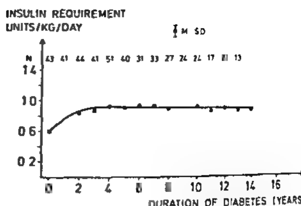


Fig 8 Daily insulin requirements in units per kg bod weight of 99 juvenile diabetics in relation to duration diabetes

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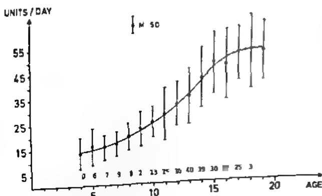


Fig 7 Daily insulin requirements of 99 juvenile diabetics of different age and sex

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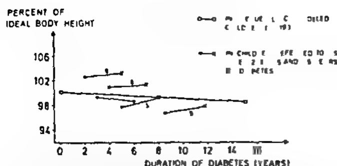


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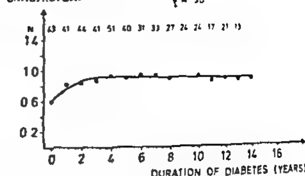


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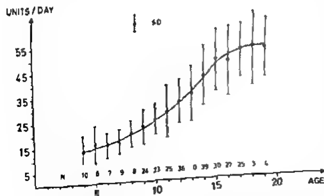


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Acta Pædiatr Scand 67 459–463 1978

VITAMIN E REQUIREMENTS OF PRETERM INFANTS

L JANSSON L HOLMBERG B NILSSON and B JOHANSSON

From the Departments of Paediatrics and Clinical Chemistry University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Jansson L Holmberg L Nilsson B and Johansson B (Departments of Paediatrics and Clinical Chemistry University of Lund Malmo General Hospital Malmo Sweden) Vitamin E requirements of preterm infants Acta Paedıatr Scand 67 459 1978 —Differences between feeding practices in earlier investigations prompted the present study of iron and vitamin E supplementation in breast milk fed preterm infants A new and highly sensitive technique for quantitation of alpha tocopherol in serum was used Studies on 34 infants with a birth weight below 2000 g or gestational age ≤35 weeks showed that supplementation with 16 5 mg tocopheryl acetate/day from 10 days of age resulted in a significantly higher haemoglobin concentration and lower reticulocyte count at 8–10 weeks than supplementation with 1 5 mg/day ($p<0$ 05) Studies on [illegible] infants with a birth weight of 2000–2499 g revealed subnormal alpha tocopherol levels in 2 of the infants given 1 5 mg tocopheryl acetate/day but there was no effect on the haemoglobin concentration at 8–10 weeks There were no untoward effects of an early iron supplementation with 2–3 mg Fe (as ferrous succinate)/kg/day It is concluded that extra supplementation with vitamin E is advisable also in breast milk fed preterm infants A low dosage iron supplementation from 3 weeks of age is safe

KEY WORDS Vitamin E breast milk preterm

Vitamin E deficiency as a cause of haemolytic anaemia in premature infants was reported by Oski & Barness in 1967 (13) Such a relation has been confirmed by some workers (8 11) but not by others (14 15) This lack of unanimity may be due to several facts The colorimetric method hitherto used for quantitative determination of vitamin E (16) has in our hands not proved reliable especially not in the analysis of small blood volumes Furthermore it is difficult to compare results from different centres because of differences in feeding practices with respect to other nutrients especially polyunsaturated fatty acids iron folic acid and selenium (5)

This study was designed to find the optimal dosage of vitamin E for preterm infants relative to iron intake In contrast to most previous studies all the infants were fed breast milk until they weighed at least 2100 g Serum alpha tocopherol levels were monitored with a newly developed and very sensitive high pressure liquid chromatography technique (12)

PATIENTS AND METHODS

Fifty seven infants with a birth weight of less than 2500 g were studied All the infants were born in Malmo General Hospital between December 1975 and February 1977 The gestational age of each infant was assessed from a combination of maternal data and the external characteristics (18) and from a neurological evaluation (1) Infants with haemoglobin values below 150 g/l or above 260 g/l in the first 24 hours were not accepted Neither were those with hyperbilirubinaemia or those who required blood transfusions Two children with mild respiratory distress were accepted None of the other children had any disease during the period of study

The infants were assigned to one or the other of 2 categories according to both birth weight and gestational age Those with a birth weight of 1000–1999 g or gestational age <35 weeks were assigned to category A those with a birth weight of 2000–2499 g in category B The infants in each category were then randomly distributed among 3 groups given different therapeutic regimens

1 Ferrous succinate (Ferromyn S AB Hassle) (2–3 mg Fe /kg/day) from 3 weeks of age (conventional regimen)

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Table 2 *Haematological data at 8–10 weeks in preterm infants given different therapeutic regimens*

Serum α tocopherol was determined at 5–6 weeks in category A and at 2–3 weeks in category B

	Category A (1 000–1 999 g)			Category B (2 000–2 499 g)		
Therapeutic regimen	1	2	3	1	2	3
Haemoglobin g/l mean (S D)	97 3 (10 [illegible])	105 3 (8 4)	98 7 (5 3)	111 2 (14 2)	113 8 (6 9)	107 0 (7 5)
Reticulocytes × 10⁹/l mean (S D)	77 7 (36 7)[b]	49 2 (29 3)	45 8 (35 4)	27 6 (16 [illegible])	36 6 (28 6)	38 8 (25 9)
Platelets × 10⁹/l mean (S D)	375 (166)	391 (185)	460 (195)	401 (165)	470 (224)	412 (213)
α tocopherol µmol/l mean (S D)	17 8 (5 0)	29 1 (5 0)	30 9 (9 9)	19 7 (7 9)[d]	26 5 (7 9)	31 3 (7 3)

$p<0.01$ compared to group 2
[b] $p<0.05$ compared to group 2
$p<0.001$ compared to groups and 3
[d] $p<0.001$ compared to groups 2 and 3

weeks of age however the groups did not differ from one another in haemoglobin level reticulocyte or platelet count

DISCUSSION

Vitamin E deficiency in prematurely born infants can be attributed to several factors The placental transfer of tocopherol is low and the tissue stores at birth are limited (3) In the most immature infants the absorption of tocopherol is impaired (11) probably because of a reduced synthesis of bile salts (19)

The function of vitamin E in the body is obscure It is thought to have an in vivo antioxidizing effect (7) which could explain the phenomena observed in premature infants Melhorn & Gross (10) found the haemoglobin to be decreased after administration of large doses of medicinal iron Williams et al (20) found a deleterious effect on haemoglobin values when they fed premature infants a formula high in linoleic acid and iron A large amount of iron would increase the oxidant stress on the erythrocyte membrane Furthermore a large amount of polyunsaturated fatty acids in the diet would increase erythrocyte linoleic acid (6) and thus the amount of oxidizable substrate This would result in an increased fragmentation of the erythrocyte membrane and even a normal level of alpha tocopherol would sometimes not be sufficient to prevent red cell destruction under such conditions

A second major biological antioxidant is glutathione peroxidase recently identified as a selenium dependent enzyme (7) Cow s milk is poor in selenium as it contains only half the amount of selenium in breast milk (9) Preterm infants fed a cow s milk formula for more than 60 days show a marked decline in plasma selenium levels as well as in erythrocyte glutathione peroxidase activity (5) The relation between this relative selenium deficiency and haemolytic anaemia of the premature infant is still not clear However with reference to the above observations the ideal formula should be high in selenium and tocopherol low in linoleic acid and iron Human milk should meet these requirements better than any commercial formula

In contrast to most previous studies the in

Table 1 *Distribution of the infants among the birth weight categories and the therapeut regimens*

The table shows birth weight gestational age and haematological data at birth

	Category A (1 000–1 999 g)			Category B (2 000–2 499 g)		
Therapeutic regimen	1	2	3	1	2	3
No of patients	13	12	9	11	5	7
Mean birth weight gram	1 899	1 767	1 866	2 135	2 330	2 258
Mean gestational age weeks	34 6	35	33 8	37 5	36 4	37
Mean haemoglobin g/l	189	194	181	207	177	192
Mean reticulocyte count $\times 10^9$/l	147	109	166	124	122	143
Mean platelet count $\times 10^9$/l	169	151	181	213	202	217

mean birth weights gestational ages and haematological data at birth did not differ between the groups From 10 days of age all infants received a multivitamin preparation (Protovit Roche) (1/2 ml/day) containing vitamins A C D the B complex and a small amount of tocopheryl acetate (1 5 mg/day) and also Leucovorin (Lederle) containing 50 μg tetrahydrofolic acid/day

The feeding routines were the following All infants were given human pasteurized milk for the first 2 weeks or until they weighed ≥2 100 g They were then changed to the commercial formula Milkotal (AB Findus) with a tocopherol content of 5 mg/l and an iron content (Ferric orthophosphate) of 12 mg/l The children were examined haematologically within the first 24 hours and again at the age of 8–10 weeks Blood for determination of tocopherol was drawn by venipuncture at approximately 5–6 weeks in category A and at 2–3 weeks in category B Sera were stored at −70 C until analyzed Haemoglobin concentration and reticulocyte and platelet counts were determined with conventional methods

Serum alpha tocopherol was determined by high pressure liquid chromatography (12) The equipment consisted of a Waters model ALC/GPC 204 liquid chromatograph a U 6K injector a model 440 UV spectrophotometer and a Corasil 1 straight phase column The absorbance at 254 nm was recorded To each serum sample (200 μl) equal volumes of 99 5% ethanol and *n* hexane were added The ethanol contained 26 6 μmol/l *dl* alpha tocopheryl acetate as an internal reference No interfering peaks could be visualized in the spectra of serum samples not containing the internal reference After careful cyclomixing the proteins were separated from the organic layer by centrifugation The hexane phase was injected directly to the Corasil column

RESULTS

In birth weight category A (1 000–1 999 g) the therapeutic groups were found to differ with respect to alpha tocopherol level as well a *haemoglobin level and reticulocyte cou* (Table 2) Serum alpha tocopherol was signif cantly lower in group 1 (low supplementa vitamin E Fe^{++} *from 3 weeks*) than in grou 2 or 3 ($p<0\ 001$) In group 1 two infants h alpha tocopherol levels below 11 6 μmol/l (tl critical lowest level according to previo studies) These 2 infants had birth weights 1 540 and 1 320 g respectively In groups 2 a 3 no infant showed an alpha tocopherol co centration below 11 6 μmol/l There was difference between groups 2 and 3

In group 1 the haemoglobin concentratio was significantly lower and the reticulocy count significantly higher than in group (high dose vitamin E Fe^{++} from 3 week ($p<0\ 05$) In group 3 (high vitamin E Fe from 10 weeks) the mean haemoglobin co centration was lower than in group 2 but t difference was not statistically significa ($0\ 05<p<0\ 1$) The platelet count did not di fer between the groups

In birth weight category B (2 000–2 499 g) th serum alpha tocopherol levels were signif cantly lower in group 1 than in group 2 or ($p<0\ 001$) In group 1 two of the infant showed serum alpha tocopherol values belo 11 6 μmol/l The birth weights of these infant were 2 280 and 2 100 g respectively At 8–1

Table 2 *Haematological data at 8–10 weeks in preterm infants given different therapeutic regimens*

Serum α tocopherol was determined at 5–6 weeks in category A and at 2–3 weeks in category B

Therapeutic regimen	Category A (1 000–1 999 g)			Category B (2 000–2 499 g)		
	1	2	3	1	2	3
Haemoglobin g/l mean (S D)	97 3 (10 2)	105 3 (8 4)	98 7 (5 3)	111 2 (14 2)	113 8 (6 9)	107 0 (7 5)
Reticulocytes ×10⁹/l mean (S D)	77 7 (36 7)[a]	49 2 (29 3)	45 8 (35 4)	77 6 (16 8)	36 6 (28 6)	38 8 (25 9)
Platelets ×10⁹/l mean (S D)	375 (166)	391 (185)	460 (195)	401 (165)	420 (224)	412 (213)
α tocopherol μmol/l mean (S D)	17 8 (5 0)	29 1 (5 0)	30 9 (9 9)	19 7 (7 9)[d]	26 5 (2 9)	31 3 (7 3)

$p<0.05$ compared to group 2
$p<0.05$ compared to group 2
$p<0.001$ compared to groups 2 and 3
$p<0.001$ compared to groups 2 and 3

weeks of age however the groups did not differ from one another in haemoglobin level reticulocyte or platelet count

DISCUSSION

Vitamin E deficiency in prematurely born infants can be attributed to several factors The placental transfer of tocopherol is low and the tissue stores at birth are limited (3) In the most immature infants the absorption of tocopherol is impaired (11) probably because of a reduced synthesis of bile salts (19)

The function of vitamin E in the body is obscure It is thought to have an in vivo antioxidizing effect (7) which could explain the phenomena observed in premature infants Melhorn & Gross (10) found the haemoglobin to be decreased after administration of large doses of medicinal iron Williams et al (20) found a deleterious effect on haemoglobin values when they fed premature infants a formula high in linoleic acid and iron A large amount of iron would increase the oxidant stress on the erythrocyte membrane Furthermore a large amount of polyunsaturated fatty acids in the diet would increase erythrocyte linoleic acid (6) and thus the amount of oxidizable substrate This would result in an increased fragmentation of the erythrocyte membrane and even a normal level of alpha tocopherol would sometimes not be sufficient to prevent red cell destruction under such conditions

A second major biological antioxidant is glutathione peroxidase recently identified as a selenium-dependent enzyme (7) Cow s milk is poor in selenium as it contains only half the amount of selenium in breast milk (9) Preterm infants fed a cow s milk formula for more than 60 days show a marked decline in plasma selenium levels as well as in erythrocyte glutathione peroxidase activity (5) The relation between this relative selenium deficiency and haemolytic anaemia of the premature infant is still not clear However with reference to the above observations the ideal formula should be high in selenium and tocopherol low in linoleic acid and iron Human milk should meet these requirements better than any commercial formula

In contrast to most previous studies the in

fants in our series received breast milk until they had reached such a degree of matu rity that the risk of developing tocopherol dependent haemolysis was low Our patients then probably got an adequate amount of poly unsaturated fatty acids and selenium To eli minate other possible deficiencies all infants received a multivitamin preparation and tetra hydrofolic acid Thus the effects of tocopherol and iron administration could be studied in the physiological setting

The present study shows that also under op timal feeding conditions tocopherol substitu tion is warranted for preterm infants with a birth weight of less than 2000 g The amount of tocopherol in common multivitamin prep arations is not sufficient as our group 1 who got only such a preparation (supplying toco pherol in a dose of 1 5 mg/day) had a signifi cantly reduced serum tocopherol content and signs of hyperhaemolysis with a lower haemo globin concentration and a higher reticulocyte count The daily dose of 16 5 mg tocopherol given to groups 2 and 3 is in the range sug gested by Dallman (3) and seems to be quite sufficient as none of the infants in this group had critically low alpha tocopherol levels ac cording to previous experiences Even in the smallest infants a satisfactory level of alpha tocopherol was reached indicating a resorption good enough under the conditions of feeding used However, our material contained only few infants with a gestational age below 32 weeks No untoward effects of the doses of vitamin E were seen

Small preterm infants run a risk of develop ing iron deficient anaemia unless the food is supplemented with iron However early use of large doses of ferrous sulfate should be avoided because it increases the tendency to haemolysis (14) A common practice in Swe den is to start iron supplementation to preterm infants at 3 weeks of age (dose 2–3 mg/kg Fe^{++} as ferrous succinate)

In the present study there was no statis tically significant difference in haemoglobin concentration or reticulocyte count between groups 2 and 3, e g the infants who recei vitamin E and iron and those given only vi min E Furthermore iron seemed not to fluence vitamin E absorption as the alpl tocopherol levels proved equal in groups and 3

As the mean concentration of haemoglol was highest in group 2 it could be conclud that iron in the doses used at least caused harm to red cell survival If iron supplemen tion is delayed there may be a risk of iron de ciency Thus judging from the present stu there is no reason to modify the conventio iron regimen

Our findings warrant the following conc sions Breast milk fed premature infants wit gestational age of less than 35 weeks or a bi weight below 2000 g seem to benefit fr supplementary vitamin E (15 mg tocophe acetate/day) given from 10 days of age fo period of 8–10 weeks Ferrous succinate (' mg Fe^{++} kg/day) should be given from 3 we of age

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DOES OVERNUTRITION OR OBESITY DURING THE FIRST YEAR AFFECT WEIGHT AT AGE FOUR?

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ABSTRACT Sveger T (Department of Paediatrics Malmo General Hospital Malmo Sweden) Does overnutrition or obesity during the first year affect weight at age four Acta Paediatr Scand 67 465 1978.—226 of 243 infants who took part in a prospective study of nutrition and weight during the first year of life were reviewed at age 4 years 23 of 243 infants (9%) were obese on one or more controls the first year and 4 of 226 (2%) at age 4 years Only 3 of 23 infants remained obese The weight and length of the children obsese at 0–1 year of age were significantly increased at age 4 years Overnutrition occurred during the first year in 26 infants and the number of obese infants in this group was significantly increased at age 7–12 months and of overweight children during the first two years of life At age 4 however none of them were either obese or overweight

KEY WORDS Obesity overnutrition

Childhood obesity may originate in infancy and the data on the natural history of obesity substantiates a significant tendency for obese children to become obese as adults (3) The adipocyte proliferation theory suggests that the prevention of overfeeding and obesity in infancy might favourably influence the prevalence of obesity later in life and that the multiplication of fat cells might be particularly sensitive to nutritional influences at certain critical stages (2)

The present study was undertaken to determine the influence of overnutrition and obesity in infancy upon obesity and overweight in 4 year-old children The measurements of weight and length recovered in the nutritional survey in infancy have been published (7)

MATERIAL AND METHODS

43 Swedish children born after 38 weeks gestation were followed prospectively at two welfare clinics Dietary intake was recorded by the 24-hour recall method (5) Weight and length were measured on average 4 times the first year (7) The weight and length were recorded for 13 children at years and for 226 (93%) at 4 years of life

Weight was measured in underpants to the nearest 0 010 kg during the first year and 0 10 kg above age 1 year Supine *length* was measured with a measuring table from birth to 1½ years and with the child against the wall with a wooden measuring rod reading in millimetres at age 2–4 years with an accuracy of 0 5 cm

Obesity was defined by comparing actual weight with that expected for age and height (4) Swedish reference standards were used (1) The index (Ix) was calculated as follows

$$\frac{\text{Actual wt (kg) of child at present age}}{\text{Actual length (cm) of child at present age}} = A$$

$$\frac{\text{50th } P \text{ expected wt (kg) for corresp age}}{\text{50th } P \text{ expected length (cm) for corresp age}} = B$$

$$\frac{A}{B} \times 100 = \text{Index (Ix) for surveyed infant}$$

An Ix above 120 indicates obesity 111–120 overweight and 91–110 normal weight

Overnutrition in this study was defined as a calorie intake exceeding the mean daily calorie consumption for infants of the same age and sex as the survey infant +2 S D The normal calorie requirement was calculated from the results of the previous study (7)

RESULTS

23 of 243 infants (9%) were obese on one or more occasions during the first year (3 of them only at birth) 11 of 213 (5%) at age 2 and 4 of 226 (2%) at age 4 years The distribution of

Table 2 *The weight index (Ix) of children with overnutrition recorded on one or more occasions in infancy compared with children on a normal calorie intake during their first year of life*
A=children with overnutrition the first year of life B=children on a normal diet the first year of life

Childhood weight status	Age							
	1–6 months		7–12 months		2 years		4 years	
	A	B	A	B	A	B	A	B
No examined	26	213	26	215	25	188	24	202
No (%) of examined children with Ix								
>120	1 (4)	13 (6)	3 (12)	7 (3)	2 (8)	9 (5)	[illegible]	4 (2)
>110–≤120	9 (35)	36 (17)	7 (27)	22 (10)	3 (12)	13 (7)	0	10 (5)

$p<0.001$

school age when children are at their leanest and almost certainly the index as calculated leaves out some obese subjects (6)

The prevalence of obesity among the survey infants was low compared with the experience of others studying this age group (6 8) At age 4 years 2% were obese and another 5% were overweight The frequencies of obese and overweight preschool children in the comparative English study was 6 9% and 11 8% respectively (6) Only 3 of 23 children who were obese as infants remained so at age 4 years but 3 of 4 obese at that age had been so since their first year The results confirm the findings made by others (6) The children who were obese in infancy had however a significantly increased length and weight at age 4 years

The mean calorie intake during the first year was close to that recommended (7) The definition of overnutrition corresponds to a calorie intake 25–30% above the calorie content of the diet recommended per kg body weight and day for a child of normal size (9) The infants with probable overfeeding during a part of infancy run a considerable risk of becoming obese or overweight the first two years of life At four years however none of them were obese and the number of overweight children who had been overfed was even lower compared with the children on a normal diet during their first year Occasional overfeeding during infancy does not appear to have any influence upon obesity in 4 year old children

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Table 1. *The mean weight and length ±1 S.D. at 4 (mean 4 3/12) years of life*
A=all survey children. B=children obese or overweight at age 4 years. C=children obese at age 0–1 year. D=children overfed at age 0–1 year

	No. examined		Length cm Mean ± 1 S.D.		Weight kg Mean ± 1 S.D.	
	♂	♀	♂	♀	♂	♀
A	118	108	107.0±3.7	106.0±4.3	17.9±1.8	17.4±2.0
B	8	7	109.6±2.2[a]	109.9±3.6[b]	21.5±1.0[b]	22.0±3.6[b]
C	10	13	109.5±2.1	109.0±2.9[b]	20.0±1.7[b]	20.1±2.4[b]
D	15	9	108.2±3.9	107.1±1.4	17.9±1.4	18.0±1.8

[a] $p<0.01$. [b] $p<0.001$.

the indexes of the 24 children who were obese on one or more visits during the first four years of life are shown in Fig. 1.

The weight and length at age 4 years of the survey children and those obese or overfed during the first year of life are given in Table 1. The weight and length of the children who had been obese during the first year and of those who were obese or overweight at 4 years were significantly increased.

Overnutrition was recorded in 26 infants on one or more occasions during the first year. In the overfed group compared with infants on a normal diet, one found at age 1–6 months a greater number of overweight infants ($p<0.001$), at age 7–12 months of overweight ($p<0.001$) and obese ($p<0.001$) infants, and at 2 years of overweight children ($p<0.001$) as shown in Table 2. 24 of the initial 26 children overfed in infancy were then seen again at 4 years of age and at that time none of them was either obese or overweight. The number of overweight children in this group was in fact lower ($p<0.001$) than among the infants on a normal diet the first year of life (Table 2).

DISCUSSION

This investigation deals with two particular questions concerning childhood obesity. The first is whether obesity in infancy disposes to obesity in preschool children, the second is the extent to which overfeeding of babies initiates excess weight gain and subsequent obesity later life.

The judgement whether the children shou be regarded as obese or overweight was ma by the index used in our previous infant stu This practical index correlates with height age and accordingly a tall child tends to classed as overweight. The survey infants w were obese were tall at 4 years of age but mo than 80% were no longer too heavy in relati to their height. The index chosen to deline obesity (120) may be too generous at the p

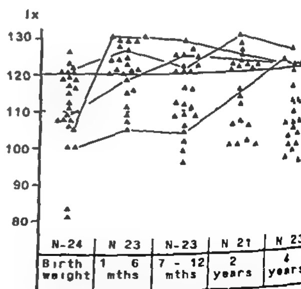


Fig. 1. The index (Ix) of the children who were obese one or more of the controls from 1 month to 4 years age. The curves showing index for the 4 children obe at age 4 years.

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ON CEREBRAL INFARCTION IN CHILDHOOD AND ADOLESCENCE

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ABSTRACT Blennow [redacted] Cronqvist S Hindfelt [redacted] and Nilsson O (Departments of Neurology Neuroradiology and Paediatrics University Hospital Lund Sweden) On cerebral infarction [redacted] childhood and adolescence Acta Paediatr Scand 67 469 1978.—This report is based on a retrospective analysis of clinical and angiographic findings [redacted] [redacted] children and adolescents suffering from cerebral infarction They were all examined during the acute stage and selective angiography was performed within a day or two of the stroke Pathogenesis [redacted] discussed and focuses particularly on the occurrence of segmental arteritis from unknown (infectious) aetiology

KEY WORDS Cerebral infarction childhood adolescence arteritis infectious aetiology

Cardiovascular disease developing with age is the common denominator of most stroke prone patients Consequently ischaemic stroke in childhood and adolescence is rare but its occurrence in a previously healthy child often poses intriguing questions about pathogenesis Various reports have dealt with stroke in the young (1 5 8 11 12) and certain predisposing factors have been delineated applying to singular cases (4) However in most instances the immediate cause remains enigmatic

The rarity of cerebral infarction early in life and our lack of knowledge about aetiological factors necessitates extensive documentation of such cases Accordingly we want to present our experiences from a minor series of young stroke patients

MATERIAL AND METHODS

Over a 14 year period (1963 to 1977) 14 patients suffering from acute cerebral infarction were cared for at the Departments of Neurology Neurosurgery and Paediatrics University Hospital Lund and the report is based on a retrospective analysis of the ischaemic stroke pattern in these young patients The series do not represent a true statistical sample since patients with cerebral infarctions secondary to preictally recognized congenital or acquired heart conditions were excluded

The diagnosis of cerebral infarction was ascertained from the history the neurological and laboratory findings and confirmed by selective angiography usually performed within 48 hours after the onset of the stroke

The 14 patients 11 males and 3 females were all below the age of 20 years with a mean of 12 2 years (median 12 years range 2 5 [redacted] 19) After the acute stage the patients have been followed on an outpatient basis

RESULTS

Stroke development and the angiographic findings are summarized in Table 1 Certain features and patterns are noteworthy The impending ischaemic stroke was never heralded by transient neurological defects (TIAs) commonly encountered among older patients In 5 cases headache was reported as a remarkable symptom occurring for days or even weeks before the onset of the stroke

The onset of stroke was typically acute occurring during activity In most cases the neurological deficits were maximal at the onset though in some patients the symptoms progressed markedly during the following hours Consciousness was usually retained except in 3 cases (G L P L and O L) all of whom had vascular occlusions within the left middle cerebral artery or the adjacent carotid siphon

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Table 1 *Stroke development and angiographic findings*

	Stroke development	Angiographic findings
1 ♂ M J 670811 age 13 y	Headache during preceding weeks Gradual onset of right sided hemiparesis and a hemisensory defect	Bilateral carotid and left sided vertebral angiograms on day 2 occlusion of left post rior cerebral art Evidence of multiple occlusions within both middle cerebral arteries *Interpretation* emboli
2 ♂ G I 600410 age 7 y	Awoke with gradual right sided hemiparesis Subsequent deterioration within hours with loss of consciousness Clinical signs indicating left sided supratentorial lesion though bilateral extensor plantar responses	Bilateral carotid and left sided vertebral angiograms on day 2 irregular stenosis peripherally in left middle cerebral artery and multiple occlusions of branches of Sylvian vessels No shift of midline vessels *Interpretation* stenosis and emboli
3 ♂ N A 720704 age 2 y	Serous meningitis diagnosed 8 days prior to the stroke Sudden onset of right sided focal motor epilepsy sustained for hours Afterwards left sided hemiparesis was noted	Bilateral carotid angiograms on day 3 On the right an intraluminal filling defect in the carotid siphon *Interpretation* embolus
4 ♂ R N 640823 age 12 y	Headache the days preceding stroke Sudden onset of right sided hemiparesis	Left sided carotid and vertebral angiograms on day 1 first part of left posterior cerebral artery is slightly irregular Occlusion of calcarine artery *Interpretation* stenosis and embolus
5 ♀ A J 520420 age 11 y	No prodromes Sudden onset of left sided hemiparesis Drowsy Deteriorated within hours to complete left sided hemiparalysis hemisensory and visual field defects	Bilateral carotid and left sided vertebral angiograms left carotid and vertebral angiograms were normal Right sided carotid angiograms—see Fig 2a b c *Interpretation* arteritis
6 ♂ O L 540922 age 16 y	Drug addict Preceding headache and difficulty in swallowing Awoke and felt had suddenly lost consciousness developed right sided hemiparalysis Regained consciousness 2 days later completely aphasic	Bilateral carotid and left sided vertebral angiograms on day 1 a first left sided carotid angiogram was technically inadequate but internal carotid artery was patent When repeated later on same day an occlusion was seen in left carotid siphon *Interpretation*
7 ♂ G N 480820 age 19 y	Headache for several hours prior to stroke He was found confused with right sided hemiparesis and hemisensory defect plus a homonymous visual field defect	Left sided carotid and vertebral angiograms on day 15 and 20 respectively the left post cerebral art gradually tapered off in peripheral direction with a complete occlusion after 1 5 cm *Interpretation* arteritis
8 ♀ A P 531220 age 16 y	While infected (common cold) she suddenly noticed weakness in her right leg The condition progressed within minutes to a complete hemiparalysis with marked aphasia	Bilateral carotid angiograms on day 1 On left side occlusions of the peripheral branches of superficial temporal art stenosis noted with slow and incomplete filling of vessel *Interpretation* embolus
9 ♂ P L 540408 age 9 y	Earlier complaints of headache Sudden onset of right sided hemiparesis including a hemisensory defect Probably a transient loss of consciousness No seizures observed	Bilateral carotid angiograms on day 1 On left occlusions of peripheral branches of superficial temporal and angular arteries Stenosis seen within first mentioned vessel with slow and incomplete peripheral filling *Interpretation* embolus
10 ♂ A E 650528 age 12 y	While playing he suddenly suffered from headache and a left sided weakness Examination revealed a left sided hemisensory defect as well Progressive deterioration 2 days later consciousness was lost and the patient exhibited signs of brainstem failure	Right sided carotid angiogram on day 1 multiple concentric narrowing of carotid siphon extending into middle and anterior cerebral arteries Multiple occlusions within branches of the pericallosal and middle cerebral arteries *Interpretation* fibromuscular dysplasia

Table 1 *(cont.)*

	Stroke development	Angiographic findings
11 ♀ M M 710107 age 6 y	Vague premonitory symptoms When she awoke after a nap she could not use her right arm properly Marked hemiparesis developing during next few hours	Left sided carotid angiogram on day 2 occlusions of multiple small arteries from posterior parietal and frontal ascendant arteries Filling defects noted in other vessels Local slowing of circulation *Interpretation* embolus
12 ♂ V O 670115 10 y	No prodromes Sudden inability to use his right arm subsequently dragging his right leg with difficulty when walking	Left sided carotid angiography about 2 weeks after onset local narrowing within middle cerebral artery close to carotid siphon—length 15 mm *Interpretation* arteritis
13 ♂ B E 510512 age 19 y	At the time of stroke he suffered from a sore throat and high fever due to infectious mononucleosis Sudden right sided hemiparalysis aphasia visual field defect	Left sided carotid angiograms on days 1 and 7 occluded internal carotid artery When repeated the carotid artery was patent Abnormal circulation within Sylvian vessels *Interpretation* embolus
14 ♂ R L 4908 age 19 y	No prodromes Sudden left sided hemiparalysis	Left sided carotid angiogram on day 1 occlusion close to bifurcation of internal carotid artery *Interpretation* embolus

Another 2 patients (G N A J) suffering from infarctions within the territories of the right middle cerebral and the left posterior cerebral arteries were slightly obtunded at the onset During the acute stage focal epileptic seizures ipsilateral to the infarcted area occurred in 2 patients (N A A E) indicating possible involvement of both cerebral hemispheres

The neuroradiologist s (S C) interpretation of the angiograms are given in Table 1 In all instances the carotid bifurcations at the level of the common carotid artery were normal and in the cases examined by vertebral angiography the subclavian origin of the vertebral artery was free from changes By radiological criteria most patients (nine) exhibited singular or multiple emboli In 2 of these the pattern was that of a proximal stenosis and multiple distal occlusions In another 3 patients a diagnosis of arteritis was entertained yet another was diagnosed as suffering from fibromuscular dysplasia In one case (O L) no definite radiological diagnosis was stated This patient had clinical evidence of an infarction within the left cerebral hemisphere A first angiogram was inconclusive though patency of the left carotic artery could be documented On repeated angiography later the same day an occlusion within the left carotid siphon was demonstrated

Aetiology

The clinical characteristics of stroke in the young do not altogether conform with those in the elderly This also applies to the occurrence of various predisposing factors (9) No patient in this series suffered from diabetes nor from manifest hypertension The fasting levels of serum cholesterol and triglycerides were determined in 8 patients in 2 of whom the cholesterol exceeded 6 4 mmoles/l The triglycerides were consistently within normal range Family history was scrutinized for predisposing factors One patient had an aunt suffering from diabetes her parents were in perfect health One patient with possible hyperlipidemia had had three grandparents who had succumbed to cardiovascular disease The mother of a third child suffered from multiple sclerosis In all other respects family history was unrevealing

Many of the patients were considered to suffer from embolic stroke Such a diagnosis im

Table 1 *Stroke development and angiographic findings*

	Stroke development	Angiographic findings
1 ♂ M J 620811 age 13 y	Headache during preceding weeks Gradual onset of right sided hemiparesis and a hemisensory defect	Bilateral carotid and left sided vertebral angiograms on day 2 occlusion of left posterior cerebral art Evidence of multiple occlusions within both middle cerebral arteries *Interpretation* emboli
2 ♂ G I 600410 age 7 y	Awoke with gradual right sided hemiparesis Subsequent deterioration within hours with loss of consciousness Clinical signs indicating left sided supratentorial lesion though bilateral extensor plantar responses	Bilateral carotid and left sided vertebral angiograms on day 2 irregular stenosis peripherally in left middle cerebral artery and multiple occlusions of branches of Sylvian vessels No shift of midline vessels *Interpretation* stenosis and emboli
3 ♂ N A 720704 age 2 y	Serous meningitis diagnosed 6 days prior to the stroke Sudden onset of right sided focal motor epilepsy sustained for hours Afterwards left sided hemiparesis was noted	Bilateral carotid angiograms on day 2 On the right an intraluminal filling defect in the carotid siphon *Interpretation* embolus
4 ♂ R N 640823 age 12 y	Headache the days preceding stroke Sudden onset of right sided hemiparesis	Left sided carotid and vertebral angiograms on day 1 first part of left posterior cerebral artery is slightly irregular Occlusion of calcarine artery *Interpretation* stenosis and embolus
5 ♀ A J 520420 age 11 y	No prodromes Sudden onset of left sided hemiparesis Drowsy Deteriorated within hours to complete left sided hemiparalysis hemisensory and visual field defects	Bilateral carotid and left sided vertebral angiograms left carotid and vertebral angiogram were normal Right sided carotid angiograms—see Fig 2*a b c* *Interpretation* arteritis
6 ♂ O I 540922 age 16 y	Drug addict Preceding headache and difficulty in swallowing Awoke and felt bad suddenly lost consciousness developed right sided hemiparalysis Regained consciousness 2 days later completely aphasic	Bilateral carotid and left sided vertebral angiograms on day 1 a first left sided carotid angiogram was technically inadequate but internal carotic artery was patent When repeated later on same day an occlusion was seen in left carotid siphon *Interpretation* ?
7 ♂ G N 480920 age 19 y	Headache for several hours prior to stroke He was found confused with right sided hemiparesis and hemisensory defect plus a homonymous visual field defect	Left sided carotid and vertebral angiograms on day 15 and 20 respectively the left post cerebral art gradually tapered off in peripheral direction with a complete occlusion after 1.5 cm *Interpretation* arteritis
8 ♀ A I 531220 age 16 y	While infected (common cold) she suddenly noticed weakness in her right leg The condition progressed within minutes to a complete hemiparalysis with marked aphasia	Bilateral carotid angiograms on day 1 On left side occlusions of the peripheral branches of superficial temporal art stenosis noted with slow and incomplete filling of vessel *Interpretation* embolus
9 ♂ P I 540408 age 9 y	Earlier complaints of headache Sudden onset of right sided hemiparesis including a hemisensory defect Probably a transient loss of consciousness No seizures observed	Bilateral carotid angiograms on day 1 On left occlusions of peripheral branches of superficial temporal and angular arteries Stenosis seen within first mentioned vessel with slow and incomplete peripheral filling *Interpretation* embolus
10 ♂ A E 630528 age 12 y	While playing he suddenly suffered from headache and a left sided weakness Examination revealed a left sided hemisensory defect as well Progressive deterioration 2 days later consciousness was lost and the patient exhibited signs of brainstem failure	Right sided carotid angiogram on day 1 multiple concentric narrowing of carotid siphon extending into middle and anterior cerebral arteries Multiple occlusions within branches of the pericallosal and middle cerebral arteries *Interpretation* fibromuscular dysplasia

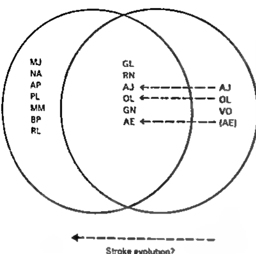


Fig 1 Hypothetical development of ischaemic stroke

the results were normal and on retesting 3 of those earlier abnormal had normalized

It should be added that all patients were subjected to single or repeated lumbar taps Routine analyses including electrophoretic protein pattern did not provide any aetiological hints

Course and outcome

One patient (A E) died on the third day after ictus exhibiting rostral–caudal deterioration and subsequent brainstem failure Autopsy confirmed the diagnosis of fibromuscular dysplasia

In most other cases recovery was early noticeable with varying degrees of restitution over the following weeks Table 2 provides a rough estimate of recovery with time and in relation to the topography of the infarct The terms minor moderate and severe neurological deficits denote monoparesis or persistent sensory defects incomplete hemiparesis without overt cognitive disturbances and hemiparalysis with defects in cognition and mentation respectively

Up to date most patients have persistent ...gical deficits though no child needs ...ursing nor technical devices for ordinary living In some cases mental development has been retarded while others have been able to fulfil education one even on university level Four patients are presently completely free from neurological defects (Table 2) A few have developed postapoplectic epilepsy but this has not added significantly to the handicaps of these patients So far none has suffered a second stroke nor symptoms indicating transient ischaemic attacks Other illnesses relevant to stroke development has not emerged

COMMENT

The difference in stroke incidence early and late in life is usually ascribed to the subsequent development of atherosclerosis and associated cardiac complications This being factual one possible implication would be that stroke in the young represents premature aging Such a concept precludes pathogenetic differences at various ages which seems untenable since manifest cardiovascular pathology in the young stroke patient is rare Consequently aetiological alternatives are warranted

The present data indicate differences between the clinical and radiological interpretations of the findings About half the patients got a diagnosis of embolic infarction based upon the angiographic findings (Table 1) The expected clinical correlate would be that of a sudden onset of the stroke as was reported in four cases (A P P L B P and R L) In a further 3 patients (M J N A and M M) with presumed embolic infarcts the onset was atypical One of the patients had troublesome headache for days or weeks before another had vague premonitory symptoms hours before Furthermore no embolic source was ever revealed nor were evidence of dissiminated embolization

In the remaining patients (Table 1) stenoses with or without peripheral occlusions were found The luminal irregularities were consistently within the intracranial vascular bed

Table 2 *Prognosis of neurological deficits with regard to vascular involvement and follow up period*

	Neurological deficits							Follow up period (years)
	At onset			At follow up				
	Minor	Moderate	Severe	None	Minor	Moderate	Severe	
Occlusion of a major artery								
O L			×			–		No follow up
N A			×			■		2 5
B P			×			×		6
R L			×	×				8
Occlusion of peripheral arteries								
M M			×		×			1
M J		×			×			2
P L		×		×				14
A P			×			×		8
Local arterial changes								
G L			×		×			1 5
R N		×		×				1 5
V O		×		×				0 5
G N		×			×			10
A J			×			×		14
A E			×	ad mortem				3 days

Denotes post apoplectic epilepsia

plicates an embolic source or clinical evidence of multiple embolization Among these patients known heart disorders were precluded and all patients were subjected to extensive cardiac evaluation by a cardiac consultant In a single case (B P Table 1) the ECG was suggestive of perimyocarditis (secondary to infectious mononucleosis) and it is possible that in this instance the stroke was due to embolization from a fragmenting mural thrombosis In the remaining patients the heart was not considered a likely source of emboli

Vascular changes i e stenoses were frequently encountered (Table 1) and since atherosclerosis can hardly be implicated these luminal irregularities may tentatively represent regional arteritis Clinical evidence of disseminated vascular disease was consistently lacking Antinuclear factor was analysed for in 5 patients in 2 of whom a radiological diagnosis of arteritis was entertained (G N V O Table 1) The results were unrevealing

On admission 4 patients exhibited overt infectious disorders (N A A P B P and O L) Of the other 10 patients 4 were subfebrile/febrile during the acute stage without obvious cause Two patients (lacking infectious symptoms had sedimentation rates consistently above 20 mm/hr and abnormal patterns of serum proteins on electrophoresis Antistreptolysin antistaphylolysin titres were determined in 6 patients with negative results In the same number of patients isolation of virus failed using faecal/urine samples and throat swab materials Three of these patients were completely void of infectious symptoms and signs

Twelve patients were subjected to extensive search for abnormalities of blood coagulation (Laboratory of Blood Coagulation Malmo) The analyses were usually not done during the first few days after the stroke Yet abnormalities were encountered in 6 Five patients exhibited a decreased fibrinolytic activity in response to venous occlusion and one an increased thrombocytic adhesivity In 6 patients

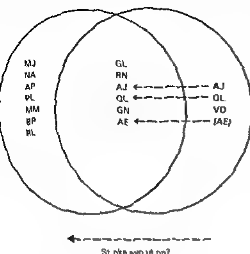


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In the remaining patients (Table 1) stenoses with or without peripheral occlusions were found The luminal irregularities were consistently within the intracranial vascular bed







Fig 2 A J Girl age 11 Right sided carotid angio graphy on the second day after onset of symptoms showe the carotid siphon to be narrowed with an irregular lume (*a*) At left sided carotid angiography on the seventh da a progression of the changes was seen (*b*) The middl cerebral artery was now occluded at the bifurcation Th carotid siphon showed increased narrowing One mont later right sided carotid angiography showed partial re canalization of the middle cerebral artery with sparse fil ling of some Sylvian vessels (*c*) Extensive narrowing o the middle cerebral artery and increased narrowing al of the carotid siphon and of the anterior cerebral artery The changes were interpretated as being due to arteritis

and did not affect extracerebral vessels In some patients arteritis was suggested in others the most likely pathology was not commented upon Retrospectively arteritis as a manifestation of disseminated collagen disorder seems highly unlikely

Despite the variable presentations of stroke (Table I) it is possible to delineate from the angiographic findings two basic morphological patterns with frequent overlappings viz 1) *stenosis* 2) stenosis plus occlusion(s) 3) *occlusion*(s) (Fig 1) Assuming a common pathogenesis these categories may represent a pathodynamic continuum—an initial vascular lesions causing a thrombotic process which results in vascular occlusion Proof would be provided by repeated angiograms at suitable intervals after the stroke although the immediate preictal period should be the ideal starting point In the present series 2 patients (O L and A J) were subjected to repeated angiographic evaluations during the early stage (Fig 2*a b c*) In these 2 cases the sudden onset of fixed neurological defects (embolic stroke)

was associated with vascular changes while occlusions could not be demonstrated at this early stage Hypothetically the vascular occlusions demonstrated later in the course may have been initiated by a primary segmental endarteritis Such a mechanism would provide a feasible explanation for the occurrence of premonitory symptoms in some patients as well as the lack of conceivable embolic source

A concept of a triggering endarteritis would also be compatible with the high incidence of concomitant infectious disorders and with the frequency of transient abnormalities of blood coagulation An endarteritis is probably systemic and affects the endothelial lining more or less uniformly on both the arterial and venous sides Such an inflammatory process might conceivably affect intravascular clotting and thus the blood coagulation data may be compatible with such an hypothesis

An infectious aetiology of stroke has recently gained support from various studies (1 6 7) but the nature of the infectious agent(s) has remained unknown A viral infection is plausible and certain Coxsackie type viruses have been shown to induce occluding arteritis (3) causing myocardial and cerebral infarctions in the young (2 10) Although certain infectious agents may have a high affinity to vascular elements the diversity of infectious syndromes associated with stroke indicates that an endarteritis may be an unspecific reaction to infection perhaps secondary to immunological events (6) Future research along these lines seems warranted

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Acta Pædiatr Scand 67 477–479 1978

GONADAL DYSFUNCTION IN PATIENTS WITH ATAXIA TELANGIECTASIA

Z ZADIK S LEVIN R PRAGER LEWIN and Z LARON

From the John Askin Laboratories Department of Pediatric Research Kaplan Hospital Rehovot and the Institute of Pediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva and Sackler School of Medicine Tel Aviv University Israel

ABSTRACT Zadik Z Levin S Prager Lewin R and Laron Z (John Askin Laboratories Department of Pediatric Research Kaplan Hospital Rehovot and the Institute of Pediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva and Sackler School of Medicine Tel Aviv University Israel) Gonadal dysfunction in patients with ataxia telangiectasia Acta Paediatr Scand 67 477 1978 —Two males and three females with ataxia telangiectasia aged from 4 9/ to 23 years were subjected to an i v LH RH test All were found to have elevated basal levels of FSH and three had elevated basal levels of LH In all the response of FSH to LH RH was supranormal In the pubertal and adult females the basal levels of estradiol were low The laboratory and clinical findings in these patients as well as data reported by others indicate that the primary gonadal failure is an integral part of AT

KEY WORDS Ataxia telangiectasia gonadotrophins gonadal dysfunction LH RH test glucose tolerance test

Ataxia telangiectasia (AT) is a syndrome characterized by progressive cerebellar ataxia oculocutaneous telangiectasia recurrent infection due to partial immune deficiency and early death from either infection or malignancy (10) In some patients glucose intolerance and hyperinsulinism have been reported (12) Glucosuria is evidently rare and ketosis has never been observed (13) Pituitary and thyroid function have been reported to be normal in these patients as have urinary steroid and gonadotropin excretion (1 10) but post mortem examination have revealed histological abnormalities in the anterior pituitary (3) and developmental abnormalities such as hypoplastic ovaries and uterus in the female reproductive system (2 3 10 11)

As part of a larger study on the relationship between the endocrine and immune systems five patients with AT known to have an immune deficiency were subjected to an i v stimulation with LH RH

SUBJECTS AND METHODS

Five patients with AT 2 males and 3 females aged from 4 / to 23 years (Table 1) were subjected to an i v LH RH test All showed the typical signs and symptoms of AT including severe ataxia difficulty in walking ocular telangiectasia recurrent pulmonary infection and immune deficiency as evidenced by impaired T cell function Three patients one of the females and both the males were prepubertal at the time of testing One of the other 2 females was already pubertal and the other had achieved maturity but in both breast development was retarded for their age and stage of pubic hair (Table 1)

The LH RH test (i v injection of 50 $\mu g/m^2$) and determination of plasma LH and FSH were performed as described in a previous report (7) All patients also underwent a 3 hour oral glucose tolerance test and tests of thyroid and adrenal function including a TRH test with determination of the plasma TSH response an i v insulin tolerance test and determination of plasma GH and the 11 OHCS steroid response all of which were performed according to standard procedures (8)

RESULTS

There was a normal response of blood glucose and plasma insulin in 4 patients in patient 5

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RESULTS

There was a normal response of blood glucose and plasma insulin in 4 patients in patient 5

Table 1 *Pertinent clinical data and laboratory investigations of the hypothalamo pituitary gonadal axis in 5 young patients with ataxia telangiectasia*
CA=chronological age Ht –height

Pat no	Sex	CA (y)	Ht (cm)	Testicular vol ml Breast dia meter cm	Pubic hair (stage 1–5)	Penis length (cm)	LH RH test 50 μg/m² iv LH IU/l[b] Basal	Peak
1	F	$4^{6}/_{12}$	100	–	1	–	1 05 (0 43±0 17)	3 85 (3 03±0 1
2	M	7	115	1 0	1	4 5	4 7 (0 54±0 1)	9 0 (1 3±0 17
3	M	10	128	1 5	1	5 5	0 25 (0 54±0 1)	1 03 (1 3±0 17
4	F	13	125	5 0	3	–	2 86 (0 93±0 1)	19 75 (9 66±0 9
5	F	23	122	8 0	5	–	0 97 (2 39±0 3)	7 74 (13 8±? I

Staging according to Tanner (8) P_1=prepubertal P_5=adult stage
[b] In parenthesis normal values (mean±1 S D) for comparable pubertal stage from Dickerman et al (11)

there was glucose intolerance and a low insulin reserve The plasma TSH response plasma GH and the response of 11 OHCS were all normal

The basal levels of plasma LH FSH and testosterone or beta estradiol and the peak LH and FSH responses of these 5 patients are shown in Table 1 Compared to norms for age and pubertal stage (4) the basal and peak re sponses of LH were elevated in 2 patients (nos 2 and 4) and at the upper limit of normal in one (no 1) In 3 patients the basal and peak responses of FSH were significantly higher than in normal children adolescents or young adults and in 2 (nos 2 and 3) they were border line high Plasma testosterone and beta estra diol were within the normal range for the pre pubertal children and low in the pubertal and adult female patients

DISCUSSION

Although hypoplasia of the ovaries or uterus has been described by several authors on the basis of post mortem findings (2 3 10 11) to the best of our knowledge no dynamic in vestigation of the hypothalamic–pituitary–gonadal axis of patients with AT has yet been reported

Three of our patients (2 males 1 female) were prepubertal their genitalia being com patible with their stage of development The elevated levels of basal plasma FSH and the increased response to LH RH in all patients even those in the stage of prepuberty strongly indicates a long standing probably primary congenital gonadal failure like that reported in other congenital syndromes such as Bloom syndrome (6) and Klinefelter s syndrome (5) in males and Turner s syndrome in females (9) The fact that the plasma LH response was elevated in only 3 patients and within normal limits for their age in the other 2 (1 male, 1 female) indicates that not all the cellular ele ments of the gonads are equally affected In the male the preponderant lesion is evidently tubular while in the female it is the ovarian follicle the latter having been histologically documented by post mortem findings (11)

It would appear very likely that primary hypogonadism is an integral part of the syn drome of ataxia telangiectasia

The glucose intolerance and low insulin out put found in one out of the five patients is in

SH IU/l			
asal	Peak	Testos (ng/100 ml)	17β E (pg/ml)
.8 (6±0 3)	35 6 (? 0±0 9)		<?0
9 (1 6±0)	5 5 (1 5±0 5)	9 1	
08 (1 6±0 ?)	4 9 (1 5±0 5)	8 5	
39 (. 4±0 5)	7 39 (3 4±1 ?)		<20
06 (.5+0 9)	11 0 (4 8±1 8)		< 0

line with previous reports (12 13) and seems to be another possible endocrine disturbance in this syndrome

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PHYSICAL HEALTH OF TEN YEAR OLD CHILDREN

An Epidemiological Study of School Children and a Follow up of Previous Health Care

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ABSTRACT Kornfält R and Kohler L (Dalby Community Health Research Centre Dalby and Department of Paediatrics University Hospital Lund Sweden) Physical health of ten year-old children An epidemiological study of school children and a follow up of previous health care Acta Paediatr Scand 67 481 1978.—At 10 years of age all 223 children in a school district underwent a physical examination and a screening for vision and hearing defects within the school health services The purpose of the study was to detect health problems of importance for the day to-day functioning of the child In 26 1% significant deviations were found Physical disorders comprised 11 7% visual defects 11 7% and auditory impairment 2 7% The vast majority of significant health problems were previously known and in only 4 4% of the 223 children newly detected 0 9% by the physical examination 2 7% by the vision screening and 0 9% by the auditory screening The most frequent health problem of all was allergy in 13 5% in 5 4% regarded as functionally important Minor orthopaedic deviations and motor disturbances were common but not often considered to affect the functioning of the child significantly As a whole the children s health was very good and the outcome of the physical examination at this age was not impressive It is evident that the physician s role in the school health system needs to be reconsidered

KEY WORDS School health physical examination vision screening auditory screening allergy motor disturbances

The importance of early recognition and prevention of diseases in childhood is well documented and has led to the application of health programme and screening tests for detection of diseases most countries The physical health of Swedish children is regularly checked by doctor's examinations during the first year of life 4–7 times from one to seven years of age once a year and after school entrance every third year This programme has been used for more than 30 years The attendance rate is over 95% (12) During this period the physical health of children has much improved The most important reason is without doubt the increase of the general socioeconomic level implying better food hygiene and housing as well as better education Many diseases caused by infection and nutritional deficiencies have been eliminated

Modern society with its social changes geographical mobility and technological advances has however created new problems for children giving rise to the new morbidity as described by Haggerty et al (8) The most prominent features of this morbidity are behaviour disorders inadequate functioning in school and adjustment problems in adolescence The health needs for children have altered and it will be the task also for paediatricians to deal with these new problems

The routines of the Preschool Health Services in Sweden have been scrutinized (12 13 14 18 28) and adjustments to the needs of the children of today have been made After the introduction of the health screening of 4 year olds physical health examinations of younger school children have become less important (13 14) The problems of older school children

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PHYSICAL HEALTH OF TEN YEAR OLD CHILDREN

An Epidemiological Study of School Children and a Follow up of Previous Health Care

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The routines of the Preschool Health Services in Sweden have been scrutinized (12 13 14 18 28) and adjustments to the needs of the children of today have been made After the introduction of the health screening of 4 year olds physical health examinations of younger school children have become less important (13 14) The problems of older school children

are however still insufficiently investigated (4 22) In this follow up study of children previously examined at 4 and 7 years of age the purpose is to describe the physical health problems of 10 year old children and to discuss the methods used by the School Health Service to detect and handle these problems

MATERIAL

All 223 children 110 boys and 113 girls born in 1965 and living in the Dalby area in Southern Sweden were examined The mean age of the children was 10 5 years ±0 31 S D The children were pupils in the fourth grade of the Primary school 105 children 46% had participated in a special health control of 4 year old children in 1969 (12) All children had been examined within the regular school health services at the age of seven

Dalby in this context includes the old communities of Dalby Genarp and Veberod which from 1974 are part of the community of Lund The population in the Dalby area was 12326 (1 1 1976)

METHODS

The examinations were made in conjunction with the compulsory class health examination prescribed in the School Health Programme They were undertaken by the school nurse and the usual school physician a paediatrician (R K) as an extended class examination according to a standardized and structurized form

The children were invited to participate by a letter to their parents The invitation was accompanied by questionnaires regarding the child s development and past and present health problems Reported significant health problems were verified by checking existing medical records and were included among previously known disorders (Tables 3–6)

Height and weight measurements were performed by the nurse using the school s standard equipment a scale on the wall with a wooden head piece and a platform balance The results were compared with a standard curve for Swedish children (30)

Puberty Signs of pubertal development were recorded by the school physician according to the method described by Tanner (29) In boys onset of puberty was recognized when testicular size was estimated as 4 ml or more compared with an orchidometer (21) (Tanner stage two or more) In girls incipient puberty was estimated on breast developmental stage of two or more

The blood pressure was measured in the morning (8–11 a m) with an aneroid manometer calibrated with a mercury manometer on the right arm immediately after the child had lain down The cuff size was 12×27 cm The mean arm circumference of the children was 20 7 cm (S D =2 1) Systolic blood pressure and diastolic (phase V) blood pressure were read to the nearest 5 mmHg (1 mmHg ≈0 133 kPa) All blood pressures were taken by the author (R K)

The physical examination included an estimation of physical development a recording of skin abnormalities spine deformities and feet abnormalities auscultation of the heart palpation of thyroid lymph nodes abdomen and in boys genital organs An evaluation of motor development was made by testing the coordination of fine and gross movements The child was observed during normal walking tiptoeing walking on the heels on the lateral borders of the feet (Fog s test (5)) and when hopping on one leg At Fog s test pronounced or unilateral supination pronation and extension or excessive grimacing were considered as a probable abnormality A test for involuntary movements was used as described by Prechtl (31) The child is asked to stand with his feet together and his head centred then to stretch out his arms with his fingers spread as wide apart as possible keeping them still for 20 seconds The child is told to close his eyes tightly and stick out his tongue Muscular twitches poor balance difficulties in coordination athetoid movements or excessive grimaces were considered as possible abnormality

Fine movements were observed when the child in rapid succession and in proper order opposed the thumb to the fingers of the same hand threaded a string drew a circle rhomb and square

Vision examination

The monocular visual acuity was tested by the school nurse with Snellen letters at a distance of five meters Children with a visual acuity of less than 1 0 (6/6) on one or both eyes and not previously detected and treated were referred to an ophthalmologist for further evaluation In Tables 5 and 7 the results of the professional examinations are given

Auditory examination

The auditory examination was made by a specially trained nurse using an audiometer (Tegner PTA A) with double earphones The testing was made at 250 500 1000 2000 4000 and 8000 cps in a quiet room A level of 20 dB was considered normal except at the lower frequencies (250 and 500 cps) where 25 dB was accepted Children with hearing impairment at two or more frequencies on the same ear were referred to the audiologist for further evaluation In the Tables 5 and 7 the results of the professional examinations are given

Screening for bacteriuria was done in girls using a test paper Uriglox® which has been proved to be suitable as a screening method for bacteriuria (6)

A classification of the health problems according to their severity or importance for the child s well being was made after a method used by Kohler (12 13) (Table 1) Deviations of groups 2 and 3 were regarded as functionally important health problems and were termed significant while the slight deviations were termed insignificant Previously known means that the health problems had already been detected and cared for before the actual investigation

Table 1 *Classification of health problems*

Method of examination	Group 0 Healthy child	Group 1 Slight deviation without importance	Group 2 Moderate deviation Treatment indicated	Group 3 Definitely handi capping disorder
General physical examination or anamnestical data		Flat feet phimosis Minor disturbances in motor develop- ment	Retentio testis MBD obesity structural scoliosis Allergy in need of medical treatment	Cerebral palsy organic heart disease
Vision examination		Myopia <−1 0 D Hyperopia <+1 5 D Astigmatism <±1 5 D Slight heterophoria	Refractive errors without amblyopia Heterophoria with complaints	Amblyopia strabismus
Auditory examination		Mild otosalpingitis cured by simple otological measures and few visits to the physician	Protracted otosalpin gitis requiring more intense therapy (adenoidectomy drainage tubes) Moderate sensorineural hearing impairments with need for follow up and control	Severe impairment requiring hearing aid or special education (training)

RESULTS

Height and weight The mean height and standard deviation was for boys 144 2 cm S D 7 12 and for girls 143 8 cm S D 7 24 A relation of weight to height (3) of more than +2 S D was defined as overweight 6 boys and 4 girls 4 5% were found in this group 2 children were obese weight/height>+3 S D

Puberty 71 boys and 35 girls had no puber tal signs 39 boys (35%) had testicular devel opment of stage 2 or more and 78 girls (70%) had breast development stage 2 or more

Blood pressure The results of blood pres sure measurements are presented in Table 2 No case of hypertension was found but bor derline values in relation to age for diastolic pressure (≥90 mmHg) (2 17 33) were ob tained in 4 cases

Table 2 *Blood pressure of 10 year old chil dren (n=223)*

	Systolic pressure (mmHg)		Diastolic pressure (mmHg)	
	Mean	S D	Mean	S D
Boys	114	8 9	66	7 3
Girls	116	10 1	67	8 0

Physical examination

In Table 3 the previously known and newly detected significant disorders are listed to gether Of the previously known disorders al lergy was the most frequent Altogether 30 children 18 boys and 12 girls had some form of allergy which however in 18 children was considered as slight and not interfering with

Table 3 *Significant physical disorders in 10 year old children (n=223)*

Diagnoses	Previously known *n*	Newly detected *n*	Sum *n*
Allergic diseases	12		12
Urinary tract in fections	2	1	3
Enuresis	4		4
Glomerulonephritis	1		1
Malformations	1		1
Epilepsy	1		1
Obesity (≥3 S D)	2		2
Skin disease	1		1
Scoliosis	0	1	1
	24	2	26

Table 4 *Allergic diseases in 10 year old children (n=223)*

	n
Rhinoconjunctivitis	7
Astma	2
Eczema	14
Gastrointestinal allergy	4
Combinations	3
	30

day to day functioning The different allergic manifestations are listed in Table 4

Medically treated urinary tract infection of significance was reported by one girl and one boy and they had undergone urological operations because of hydronephrosis and ureteral reflux Another 6 girls had been treated for urinary tract infections some years previously

Primary nocturnal enuresis which has not responded to treatment was reported in 3 boys and one girl The girl had bacteriuria when examined with Uriglox® and culture She had been treated several times for bacteriuria and had been examined with urography the result of which was normal

One boy had recurrent episodes of haematuria due to focal glomerulonephritis and was under close observation One girl was extensively handicapped by severe ichthyosis Two other children were handicapped by disease one boy had aplasia of the fibula of one leg which was more than 6 cm shorter than the other and one girl suffered from epilepsy with frequent fits in spite of drug therapy

The newly detected significant physical deviations were only two in number One girl was found to have bacteriuria and another girl had a marked structural scoliosis and was referred for brace treatment

The most frequent deviations found at the physical examination were skin abnormalities (6 cases) spine deformities (6 cases) and incoordination of fine and gross movements (15 cases) Twitching of the fingers and tongue (choreiform movements) was noticed in 9 children Seven children had difficulties in performing Fog s test The difficulties in the two mentioned tests were often combined Five boys could not hop on one leg Problems in fine finger movements were sometimes observed in combination with other motor disturbances but in 3 cases as the only deviation All these deviations were however considered as slight and not interfering with normal life

Some items in the investigation were always normal palpation of abdomen lymph nodes and thyroid One girl had a previously known heart murmur caused by a minimal ventricular septal defect One boy had phimosis in need of treatment

Table 5 *Classification of visual disorders among children at 4 7 and 10 years of age*

Classification	10 years *n*	10 years %	7 years *n*	7 years %	4 years *n*	4 years %
Group 0	188	84 3	197	88 3	93	88 5
Group 1	9	4 0	6	2 7	3	2 9
Group 2	16	7 2	10	4 5	3	2 9
Group 3	10	4 5	[illegible]	4 5	[illegible]	5 7
	223	*100*	*223*	*100*	*105*	*100*
Sum of functionally important disorders (groups 2 and 3)	26	11 7	20	8 9	9	8 6
Newly detected	6	2 7				
Previously known	20	9 0				

Table 6 *Classification of auditory impairment among children at 4 7 and 10 years of age*

Auditory disorders	10 years *n*	10 years %	7 years *n*	7 years %	4 years *n*	4 years %
Group 0	205	93 2	204	92 7	103	98
Group 1	6	2 7	9	4 0	0	0
Group 2	5	2 3	5	2 3	2	2
Group 3	1	0 5	1	0 5	0	0
Not followed up	3	1 3	1	0 5	0	0
	220	*100*	*220*	*100*	*105*	*100*
Sum of significant ear disorders	6	2 7	6	2 8	2	2
Previously known	4	1 8				
Newly detected	2	0 9				

Vision examination

Impaired vision was found in 35 of the 223 children 15 7% Eight children had newly detected refractive errors The total frequency of significant eye disorders was 11 7% The most frequent refractive errors were astigmatism in 14 children myopia in 12 and hyperopia in 8 children In Table 5 the results of the visual examination at 10 years of age are compared with the findings at 7 and 4 years of age in the same children Of the 105 4-year-old children 12 had eye disorders Four of them had improved their visual acuity by the time they were 7 years old 2 children with amblyopia and 2 with astigmatism Two children had deteriorated 1 boy with exotropia intermittens now manifested and 1 with myopia Between the ages 7 and 10 no pronounced improvement in children with amblyopia was noted but 8 cases of refractive errors were newly detected 7 cases of myopia and 1 with astigmatism Six of the children with myopia were prescribed glasses

Table 7 *Functionally important health problems in 10 year old children*

	n	%
Anamnestical data and physical examination	26	11 7
Newly detected	2	0 9
Previously known	24	10 8
Vision examination	26	11 7
Newly detected	5	2 6
Previously known	21	8 9
Auditory examination	6	2 7
Newly detected	2	0 9
Previously known	4	1 8
Total	58	6 1
Newly detected	9	4 4
Previously known	49	21 7

Auditory examination

Impaired hearing to some degree was found in 40 of the 220 screened children (18 2%) In 21 children the impairment was 25 dB in one of the lower frequencies and no further measures were taken in these cases According to the criteria 16 children were referred to the audiologist and 13 were examined Five were found to have normal hearing (over referral in 38%) The results of the auditory examination are presented in Table 7 together with the findings in the same children at 7 and 4 years of age The frequency of significant hearing impairment was almost the same at the 3 different examinations One third of the children with otosalpingitis at 10 years had the same problems at 7 years Only half of the children were examined at 4 years and 2 children with hearing impairment were then detected They had the same diagnosis at the following examinations Two children in group 2 were newly detected

both with otosalpingitis in need of treatment with drainage tubes One boy had been severely handicapped by sensorineural hypoacusis since a very early age and used a hearing aid

DISCUSSION

An analysis of health problems in children of different ages is essential for the planning of child health care Most published studies on health problems in children about 10 years of age present only the frequency of severe chronic diseases and the figures vary from 1 to 20% because of disparities in material methods and definitions (20 25)

The goal of the present study, however was to detect health problems in a wider sense to include also problems that although not classified as severe chronic diseases nevertheless have a definite impact on the day to day functioning of the child Special importance was assigned the detection of such significant disorders that are not easily detected without proper examination (13)

The identification of health problems is however of little practical value if resources for intervention are lacking In our country with its good economic social and medical resources, heavy demands on optimal health can be made and therefore also rather small deviations are considered to deserve treatment (13)

The standardized methods previously applied to 4 year and 7 year old children in the same area classifying the health problems according to their functional importance (12 13) were found to be useful also for older children

The most common chronic condition in the examined 10 year old children was *allergy* in 13 5%, in 1/3 of the cases significantly interfering with the child s daily activities

The frequency of atopic diseases in 7 year old children in another Swedish area was reported to be 15 1% (10) In North American and West European children the frequency is estimated to 12–24% (27) Allergy constitutes one third of all chronic conditions in children according to the United States Health Survey 1959–1961 (25) The methods of treatment of allergy have improved but in many cases they are still ineffective and time consuming Allergic diseases often give rise to secondary behaviour problems Fatigue irritability and restlessness are described often to affect these children (27) Therefore the problems of allergic children should be devoted more time and interest by the School Health Services

A high frequency of slight *disturbances of motor development* was observed in this study 6% almost totally confined to boys Wolff (35) reported a frequency of 10% of the choreiform twitch in normal elementary school children but in 50% it disappeared in 2 years He found a correlation between the findings of the choreiform twitch and reading and spelling difficulties in boys This has been confirmed by some examiners (9) but denied by others (1) Seven of the 15 children with motor disturbances in this study were underachievers according to their teachers A more detailed presentation of the 223 children s adjustment and performance in school is under preparation

The result of the neurological examination is probably to some extent influenced by tension and nervousness in the child None of the children could be said to have a syndrome of minimal brain dysfunction (MBD) when applying the criteria proposed by Hagberg (7) The concept of MBD is a very heterogenous one It may give an illusory appearance of understanding the child s problem which is more often caused by social problems or disturbances in family relations than by neurological damage (24)

Although functionally important *orthopaedic impairment* was found in only 2 cases minor orthopaedic deviations such as increased kyphosis or lordosis poor posture knock knees pes planus and static scoliosis were very frequent The most important orthopaedic deviation to diagnose at 10 years of age is the adolescent form of scoliosis

which usually starts insidiously at this age (34) Since a more severe form may develop later during the prepubertal growth spurt even children with insignificant scoliosis must be put under careful supervision The frequency of structural scoliosis in need of treatment in Sweden is estimated to about 0 4% (34)

Overweight was found to be less frequent at 10 years of age (4 5%) than at 7 years (7 2%) In larger series the weight/height ratio was constant between 7 and 10 years (19 26) Our overweight children had received dietary advice but no other special treatment

Bacteriuria was found in 1 girl who had nocturnal enuresis but no other symptoms of urinary tract infection The enuresis remained after treatment with chemotherapy Although asymptomatic bacteriuria (ABU) in children is usually considered as a potentially dangerous infection (11) recent studies suggest that it is a rather harmless condition with little or no risk of later complications and perhaps not requiring treatment (16)

Blood pressure measurements are not routinely performed in health examinations in Sweden Results of blood pressure measurements of different age groups of Swedish school children are only occasionally reported (32) The values obtained in this investigation do not differ significantly from those reported from the United States (33) Hypertension in children is reported to be rather common 2–3% when blood pressures above the 95th percentile are considered as hypertension (17) The prevailing concept is that juvenile hypertension almost always is secondary and most commonly associated with renal disease Primary hypertension in childhood has however been found to be more common than previously thought (17) As primary hypertension in adults implies an increased rate of morbidity and mortality it must be considered as important to identify prospective hypertensive children early because of the potential for prevention of fixed hypertension (2 17) Therefore it seems essential that regular blood pressure measurements should be introduced into the ordinary School Health Services

The frequency of *refractive errors* in 10-year old children (Table 5) was high 15 7% and 11 7% needed treatment Nine per cent were already taken care of All newly detected significant disorders were myopia confirming the well known increase of this refractive error in prepuberty (15)

The frequency of *functionally important hearing impairment* was the same as at 7 years of age No new case of sensorineural hearing loss was detected at 10 years The outcome of the auditory screening was the finding of 2 children with newly detected protracted middle ear infection and heavy hearing loss Otosalpingitis is very common in children and has a high tendency of spontaneous healing but can sometimes constitute great problems The method of audiometry however may not be the ideal one to detect these cases since it has low sensitivity and specificity (23) Our high frequency of over referral also indicates that the methods for auditory screening of school children need to be re evaluated

As evident from Table 7 a total of 26% of the 10 year old children were found to have important health problems by any of three methods of investigation physical examination vision screening and hearing screening Most defects were however previously known and being taken care of Of the significant health problems newly discovered in 4 4% of the children the visual defects were responsible for the largest part The number of newly discovered disorders by the auditory screening and physical examination was low The value of having physicians performing routine clinical health examinations at least in the lower grades must be questioned The school nurse is of course the main resource in health care A further study of her function and capacity of screening also for physical disorders is in progress and will be published separately If the doctor could be released from routine clinical examinations he could concentrate on the real problems of the school

both with otosalpingitis in need of treatment with drainage tubes One boy had been severely handicapped by sensorineural hypoacusis since a very early age and used a hearing aid

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children s physical health today, such as in creased help for chronically ill children treat ment of acute illness and health education Most of the problems are however not physi cal but social and emotional (8) The school doctor can however through his specialized and sociomedical knowledge help to solve these problems in collaboration with other members of the school health team and agen cies outside the schools

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SYNOVECTOMY AS A PROPHYLACTIC MEASURE IN RECURRENT HAEMOPHILIC HAEMARTHROSIS

Follow up of 23 Cases

O SNEPPEN H BECK and V HOLSTEEN

From the Department of Orthopaedic Surgery U Rigshospitalet University of Copenhagen Copenhagen Denmark

ABSTRACT Sneppen O Beck H and Holsteen V (Department of Orthopaedic Surgery U Rigshospitalet Copenhagen Denmark) Synovectomy as a prophylactic measure in recurrent haemophilic haemarthrosis Acta Paediatr Scand 67 491 1978 —Because of frequently recurring haemarthrosis which could not be controlled by conservative management 19 haemophiliacs were subjected to synovectomy on a total of 23 joints—17 knees 5 elbows and 1 hip The patients were followed for an average of 23 months Primary postoperative complications occurred in the form of recurrent bleeding in 8 joints Rehabilitation was often difficult and long lasting and the range of joint motion was essentially restricted in 4 cases After a follow up period exceeding 6 months the findings in the remaining mobile 19 synovectomized joints were 11 had been relieved of haemorrhage 5 had rare and two frequent haemorrhages The reduction in the number of haemorrhages was significant ($p<0\ 01$) In the light of the complicated postoperative course it is concluded that synovectomy should be used only on strict indications viz only in otherwise intractable cases of progressing haemophilic arthropathy

KEY WORDS Haemophilia synovectomy

Chronic arthropathy the most disabling component of haemophilia manifests itself mainly in cases where the Factor VIII or IX level is below 1% of normal while it is uncommon and usually less serious if the level of these factors is between 1 and 4% (1) The introduction in the late 1960s of prophylactic long term replacement therapy which made it possible to keep the factor level above the critical limit held out a hope of a better prognosis (7) It was expected that an adequately treated patient could now lead an almost normal life with considerably less risk of the disablement previously caused by chronic haemophilic arthropathy (4 9 11) According to more recent investigations however this was over optimistic True the prophylactic replacement therapy has definitely improved the prognosis (3 12) but it does not prevent the development of chronic haemophilic arthropathy in quite a large number of cases (3 10 15) Consequently supplementary therapeutic measures are still needed and this is where synovectomy comes in

From the reports on synovectomy in haemophilia which have been published so far it is apparent that in the short term i e during the first years after the operation the procedure has reduced the number of bleeding episodes (Table 1) But this agreement on the effect of synovectomy upon the bleeding tendency does not apply to the complications involved by the operation In particular several authors have encountered considerable postoperative rehabilitation problems and in a number of cases joint mobility becomes permanently restricted (13 17) Others seem to have had relatively few or no complications (6 16) (Table 1)

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s 7 and 20 had a Factor IX level 1% Case 22 Factor VIII 3% and the others Factor VIII 1%

e	Age at op	Joint	Prim postop complications (haemarthroses)	Follow up period (mos)	Mean no of bleeding episodes per month		Mobility		Patients assessment of joint status
					Pre-op	Post-op	Pre-op	Post-op	
	9	Knee	Haem 18th day	104	2–3	0	70–115	40– 45	Worse
	21	Knee	Haem 9th 14th 19th day	87	2–3	[redacted]	0–125	10– 85	Unchanged
	8	Knee	0	77	4	1	20– 50	0–110	Unchanged
	8	Knee	0	71	4	1	70–140	0–100	Unchanged
	[redacted]	Knee	0	65	4	4	5–100	0–100	Worse
	9	Knee	[redacted]	59	4	0	15– 90	10– 80	Improved
	11	Knee	Haem 6th 12th 30th day	57	4	0	10–110	0–125	Improved
	14	Knee	Haem 4th 7th day	53	4	1	10– 45	5– 75	Improved
	11	Elbow	0	43	4	1	45–130 70/70	20–12[redacted] 50/60	Unchanged
0	17	Knee	0	39	4	0	5–110	5–135	Improved
1	18	Elbow	0	32	4	0	45– 90 20/10	30–130 60/40	Improved
	16	Elbow	0	26	4	0	15–135 80/70	20–120 50/60	Improved
3	28	Knee	0	24	4	3	5– 80	30– 50	Worse
14	14	Knee	Haem 14th day	20	4	1	0–100	0– 85	Unchanged
[redacted]	18	Knee	Haem 7th 13th day	17	4	0	5–135	5– 80	Improved
16	18	Knee	Haem 14th 26th day	11	4	0	20– 30	10– 35	Improved
17	14	Knee	Haem 10th day	10	4	0	0– 95	0– 90	Improved
18	41	Knee	0	9	4	0	0–100	0–100	Improved
19	10	Knee	0	7	4	0	0– 90	0– 60	Improved
20	25	Hip	0	9	4	0	20– [redacted] 15/10	0–100 45/25	Improved
21	15	Elbow	0	3	4	0	15–135 70/80	30–100 20/20	–
22	29	Elbow	Hepatitis	3	1	[redacted]	80–100 70/60	70–100 0/35	–
23	41	Knee	[redacted]	3	2–3	0	20– 85	0– 60	–

For the knee we used a medial parapatellar incision Most emphasis was laid on removing the synovial membrane in the intercondylar notch and beneath the collateral ligaments As a rule the menisci were not removed In 3 cases [redacted] having grade IV arthropathy the knee synovectomy was supplemented with alloplasty (Cases 15 [redacted] and 18) The hip synovectomy (Case 20) was also performed on a grade IV arthropathy and combined with alloplasty At the elbow we used only the lateral approach and the head of the radius was not removed except in one case in which it was greatly deformed and in which growth in the proximal radial epiphysis had ceased (Case 22)

Replacement therapy for patients with haemophilia A was by cryoprecipitate and for haemophilia B patients a Factor IX concentrate At the time of operation the factor level was more than 50% and during the first two post operative weeks more than 25% The total dose of Factor VIII during the stay in hospital averaged about 34000 units range 19000–72000 units for the patients who had knee synovectomy and about 17000 units range 13000–27000 units for those who had elbow synovectomy After discharge factor replacement was administered in the form of 2 or 3 weekly infusions aiming at a level around 10% over a period of 2–4 months

The postoperative rehabilitation programme generally lasted for 3–6 months During the same period a protective bandage was worn

Table 1 *Results of synovectomy for haemophilic arthropathy in published reports*

Author	No of cases	Primary post operative complications	Follow up period (months)	Results
Pietrogrande et al (1972)	23	3 haemarthroses Rehabilitation difficult	6–31	11 no bleeding episodes 11 a few bleeding episodes 1 not stated 2 reduced mobility
Dyszy Laube et al (1974)	14	None	2–24	14 no bleeding episodes 0 with reduced mobility
Storti et al (1975)	63	None	6–132	38 no bleeding episodes 16 a few bleeding episodes 3 frequent bleeding episodes [illegible] reduced mobility
Schwagerl et al (1976)	9	2 haemarthroses Rehabilitation difficult	6–72	3 no bleeding episodes 6 a few bleeding episodes No information about reduced mobility
Arnold et al (1977)	10	1 haemarthroses Rehabilitation difficult	6–72	10 no bleeding episodes No information about reduced mobility
Sneppen et al (1978)	23	8 haemarthroses Rehabilitation difficult 1 hepatitis	3–104	16 no bleeding episodes 5 a few bleeding episodes 2 frequent bleeding episodes 4 reduced mobility

complications including the incidence of recurrent bleeding episodes and of impaired mobility

MATERIAL AND METHODS

During the period 1969–77 synovectomy was performed on 23 joints in 19 haemophiliacs—17 knees 5 elbows and 1 hip At the time of surgery the patients ranged in age from 8 to 41 mean 16 years This material has been followed clinically and radiologically for periods ranging from 3 to 104 months mean 23 months The most important data are listed in Table 2

All the patients but one (Case 22) had severe haemophilia with a Factor VIII and IX concentration below 1% Prior to the synovectomy the patients had had several months of conservative treatment using factor substitution non weightbearing and immobilization of the joint The only cases that came to operation were those in which a conservative regime had not led to any major reduction in the bleeding episodes Most of the patients had at the time of operation fairly advanced haemophilic arthropathy with severe synovial hypertrophy and cartilage destruction (Fig 1) According to the classification of DePalma (5) 2 of the 23 operated joints were grade II 15 were grade III and 6 were grade IV



Fig 1 Chronic haemophilic arthropathy with severe synovitis and moderate cartilage degeneration This condition had developed after 7 months serial haemorrhages and despite prophylactic factor replacement and protective bandage during the same period (Table 2 Case 19)

Table 2 *Main data in relation to 23 cases of haemophilic arthropathy treated with synovectomy*

Cases 7 and 20 had a Factor IX level 1% Case 22 Factor VIII 3% and the others Factor VIII 1%

Case no	Age at op	Joint	Prim postop complications (haemarthroses)	Follow up period (mos)	Mean no of bleeding episodes per month		Mobility		Patients assessment of joint status
					Pre-op	Post-op	Pre-op	Post-op	
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Replacement therapy for patients with haemophilia A was by cryoprecipitate and for haemophilia B patients a Factor IX concentrate At the time of operation the factor level was more than 50% and during the first two postoperative weeks more than [illegible]5% The total dose of Factor VIII during the stay in hospital averaged about 34000 units range 19000–[illegible]000 units for the patients who had knee synovectomy and about 17000 units range 13000–[illegible]000 units for those who had elbow synovectomy After discharge factor replacement was administered in the form of 2 or 3 weekly infusions aiming at a level around 10% over a period of [illegible]–4 months

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RESULTS

Table 2 lists the main therapeutic results

Primary complications

During the healing phase a total of 15 recurrent bleeding episodes occurred in 8 joints all following knee synovectomy These haemorrhages occurred from the 6th to the 30th postoperative day in 3 cases in relation to a low factor level, but in the other 12 spontaneously and in spite of adequate factor replacement Two of the haemarthroses were treated with joint puncture, 2 with arthrotomy and evacuation of a clot and the remainder merely by immobilization and increased factor replacement The haemorrhagic complications necessitated long lasting immobilization and—since these patients had fairly severe pain—this made rehabilitation particularly difficult and prolonged In 3 cases *brisement force* was needed

Late recurrent bleeding episodes

In evaluating the effect of synovectomy upon the arthropathy we excluded 3 patients who had been followed for less than 6 months (Cases 21 22 and 23) and one patient in whom follow up showed bony ankylosis (Case 1) This leaves 19 cases all with a history of frequent haemarthroses prior to the operation (Table 2) At follow up 12 of them had been relieved of bleeding episodes 5 had few and usually fairly minor haemorrhages while 2 had again developed serial haemorrhages into the operated joint after a free period of one year (Cases 5 and 13) The reduction in the number of haemarthroses calculated on the basis of the 19 cases was statistically significant ($p<0\ 01$) Incidentally Table 2 shows that this therapeutic effect was found also among the cases with a relatively long follow up period

Joint mobility

In evaluating the influence of synovectomy upon joint mobility and also in evaluating the patients own assessment of the therapeutic result we excluded (apart from the 3 cases followed for less than 6 months) 4 patients in whom synovectomy had been supplemented with alloplasty (Cases 15 16 18 and 20) This leaves 16 patients, among whom 6 had better mobility, 6 about the same mobility and 4 (Cases 1 2 13, and 19) a considerably worse mobility than prior to the operation In 3 of these cases the unsatisfactory result was due to primary postoperative bleeding and/or pain while in one it was due to a new series of bleeding episodes with progressing chronic haemophilic arthropathy

Subjective result

Among the 16 patients having a plain synovectomy and followed for more than 6 months 8 felt that their status was better 5 that it was unchanged and 3 that it was worse

DISCUSSION

Synovectomy for haemophilia is one link in a therapeutic programme including also a period of intensive factor replacement non weight bearing followed by prolonged training of joint mobility and muscles It is of particular and perhaps decisive, importance that the factor replacement therapy is carried out with fairly great intensity i e in a total dose during the operative and primary postoperative period which corresponds to that during 6–12 months conventional prophylactic replacement therapy Thus what has been recorded in the present and previously published materials is the result of a therapeutic programme and on this basis it is not possible to make a selective assessment of the synovectomy *per se*

The above items are of particular importance in materials in which the operation is done on the basis of only a few haemorrhages into the joint and not preceded by long term conservative treatment (16) and in materials in which the preceding conservative programme has not included long term replacement therapy (6) In such materials the conservative

components may play a decisive role and there is little doubt that a conservative therapeutic regime including in particular long term replacement therapy might afford healing of most of these arthropathies Even in a fairly advanced chronic haemophilic arthropathy adequate conservative treatment will afford healing of the synovitis and cessation of the recurrent haemarthrosis in about two thirds of the cases (8)

In accordance with a number of other materials subjected to synovectomy because of otherwise intractable progressing chronic haemophilic arthropathy (3 13 17) we found the treatment to have a definitely favourable effect upon the frequency of bleeding into the joint concerned We also found that the surgical procedure as such entailed a number of complications partly primary in the form of postoperative haemarthrosis pain and rehabilitation difficulties partly secondary in the form of permanently reduced joint mobility

In the light of these findings we agree with those authors who feel that synovectomy should be done only on very strict indications i e only on patients who still have haemarthrosis and thus progressing haemophilic arthropathy in spite of several months adequate conservative regime (3 14 17) Making the indications so strict may involve some risk that irreversible changes in cartilage and bone occur during the preoperative conservative treatment phase (2) (Fig 1) but in our opinion this risk is offset by the fact that the majority of cases heal during the conservative treatment and thus avoid the operation and its serious complications

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RESULTS

Table 2 lists the main therapeutic results

Primary complications

During the healing phase a total of 15 recurrent bleeding episodes occurred in 8 joints all following knee synovectomy These haemorrhages occurred from the 6th to the 30th postoperative day in 3 cases in relation to a low factor level but in the other 12 spontaneously and in spite of adequate factor replacement Two of the haemarthroses were treated with joint puncture 2 with arthrotomy and evacuation of a clot and the remainder merely by immobilization and increased factor replacement The haemorrhagic complications necessitated long lasting immobilization and—since these patients had fairly severe pain—this made rehabilitation particularly difficult and prolonged In 3 cases *brisement force* was needed

Late recurrent bleeding episodes

In evaluating the effect of synovectomy upon the arthropathy we excluded 3 patients who had been followed for less than 6 months (Cases 21 22 and 23) and one patient in whom follow up showed bony ankylosis (Case 1) This leaves 19 cases all with a history of frequent haemarthroses prior to the operation (Table 2) At follow up 12 of them had been relieved of bleeding episodes 5 had few and usually fairly minor haemorrhages while 2 had again developed serial haemorrhages into the operated joint after a free period of one year (Cases 5 and 13) The reduction in the number of haemarthroses calculated on the basis of the 19 cases was statistically significant ($p<0.01$) Incidentally Table 2 shows that this therapeutic effect was found also among the cases with a relatively long follow up period

Joint mobility

In evaluating the influence of synovectomy upon joint mobility and also in evaluating the patients own assessment of the therapeutic result we excluded (apart from the 3 cases followed for less than 6 months) 4 patients in whom synovectomy had been supplemented with alloplasty (Cases 15 16 18 and 20) This leaves 16 patients among whom 6 had better mobility 6 about the same mobility and 4 (Cases 1 2 13 and 19) a considerably worse mobility than prior to the operation In 3 of these cases the unsatisfactory result was due to primary postoperative bleeding and/or pain while in one it was due to a new series of bleeding episodes with progressing chronic haemophilic arthropathy

Subjective result

Among the 16 patients having a plain synovectomy and followed for more than 6 months 8 felt that their status was better, 5 that it was unchanged and 3 that it was worse

DISCUSSION

Synovectomy for haemophilia is one link in a therapeutic programme including also a period of intensive factor replacement non weight bearing followed by prolonged training of joint mobility and muscles It is of particular and perhaps decisive importance that the factor replacement therapy is carried out with fairly great intensity i e in a total dose during the operative and primary postoperative period which corresponds to that during 6-12 months conventional prophylactic replacement therapy Thus what has been recorded in the present and previously published materials is the result of a therapeutic programme and on this basis it is not possible to make a selective assessment of the synovectomy *per se*

The above items are of particular importance in materials in which the operation is done on the basis of only a few haemorrhages into the joint and not preceded by long term conservative treatment (16) and in materials in which the preceding conservative programme has not included long term replacement therapy (6) In such materials the conservative

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In the light of these findings we agree with those authors who feel that synovectomy should be done only on very strict indications, i.e. only on patients who still have haemarthrosis and thus progressing haemophilic arthropathy in spite of several months adequate conservative regime (3, 14, 17). Making the indications so strict may involve some risk that irreversible changes in cartilage and bone occur during the preoperative conservative treatment phase (2) (Fig. 1), but in our opinion this risk is offset by the fact that the majority of cases heal during the conservative treatment and thus avoid the operation and its serious complications.

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CLINICAL AND IMMUNOLOGICAL ASPECTS OF FOOD ALLERGY IN CHILDHOOD

II *Development of allergic symptoms and humoral immune response to foods in infants of atopic mothers during the first 24 months of life*

A DANNAEUS S G O JOHANSSON and T FOUCARD

From the Department of Paediatrics and the Blood Centre University Hospital Uppsala Sweden

ABSTRACT Dannaeus A Johansson S G O and Foucard T (Department of Paediatrics and The Blood Centre University Hospital Uppsala Sweden) Clinical and immunological aspects of food allergy II Development of allergic symptoms and humoral immune response to foods in infants of atopic mothers during the first 24 months of life Acta Paediatr Scand 67 497 1978 —36 children of atopic mothers (group I) and 17 children of healthy mothers (Group II) were selected for a study of the relationship between the onset of atopic symptoms and the occurrence of IgE- IgG and IgA antibodies with special regard to the development of antibodies to β-lactoglobulin (BLG) and ovomucoid (OM) Atopic symptoms developed in 50% of the children in group I and in no one of group II The cord serum IgE concentrations were shown to be predictive of subsequent development of atopic symptoms and the levels remained higher in group I than in group II The serum IgA concentrations were low and within normal limits in both groups and bore no relationship to atopic heredity or to later development of atopic symptoms However at six months of age the serum IgA levels were lower in the children of atopic mothers ($p<0\ 1$) Low titers of IgE antibodies to BLG and OM occurred in several children of Group I but seemed to have clinical significance in only a few children IgG antibodies to BLG and OM were shown to occur frequently and in equivalent titers in cord sera and maternal sera indicating a transport across the placental barrier High cord titers were found in children of group I who remained asymptomatic indicating a possible protective function by these antibodies The titers to BLG were higher in group I than in group II Most titers decreased during the first months of life and then a rise occurred

KEY WORDS Atopic allergy Infants IgE IgG IgA placental barrier

The risk of developing allergic symptoms to foods is greater in early infancy than later in life (7 8) The massive exposure to foreign antigens by a large intake of food proteins in relation to body weight and the increased per meability of the gut (20) explain why most in fants are more prone to develop antibodies to antigenic substances in foods than adults (18) In children with an atopic constitution this early antigenic exposure can induce the production of reagins eliciting allergic symptoms (5 15) Transferred maternal IgG antibodies to microbial agents are known to contribute to the protection of the child against infection during the first months of life (1) Whether transferred IgG antibodies to food antigens can prevent sensitization and symptoms of food allergy is not ascertained

Maternal IgE antibodies do not cross the placental barrier (2 21) IgE in cord sera is probably of fetal origin (19) and elevated levels are predictive of later atopic disease (17)

It has been suggested that a transient IgA immunodeficiency during the first months of life because of a defect protection of the GI tract could predispose to atopic disease (23) The secretory IgA in breast milk is thought to give such a protection (10 11 16)

The aim of this study was to investigate in fants predisposed to atopic disease for the de velopment of antibodies to basic food antigens

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The aim of this study was to investigate infants predisposed to atopic disease for the development of antibodies to basic food antigens

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Table 1 *Serum IgE concentrations in children of atopic mothers (Group I) and in children of healthy mothers (Group II)*

The difference was statistically significant at birth ($p<0.01$) and at 6 and 12 months of age ($p<0.05$)

Age (months)	Group I No of children	Group I IgE kU/l	Group II No of children	Group II IgE kU/l
0	36	0 24 (0 08–0 66)	17	0 11 (0 09–0 14)
3	11	1 86 (0 66–5 20)	1	1 10
6	19	5 10 (1 13–23 2)	8	[illegible] 45 (0 90–6 67)
12	25	8 91 (1 86–42 3)	7	3 46 (1 25–9 45)
18	11	6 90 (1 21–39 5)	1	2 70
24	8	10 7 (1 65–69 5)	1	5 10

* The geometric mean the geometric mean ±1 S D are indicated in parentheses

in cow s milk and egg white in relation to food antigen exposure and to clinical symptoms of allergy

MATERIALS

All mothers who attended a Mother Welfare Centre in March April and May 1974 and reported that they suffered from allergy were investigated by history Based on case history 35 atopic mothers and their thirty six new born children were included in the study (Group I) Seventeen healthy mothers without any atopic symptoms and their children selected at delivery served as controls (Group II)

At delivery a venous blood sample from all mothers and cord blood from all newborns was obtained The mothers were offered clinical investigation of their children including blood sampling at 3 [illegible] 18 and 24 months of age Children without or with slight symptoms showed a lower willingness to attend than children who got symptoms during the follow up period Thus the number of achieved blood samples varied from 1 to 6 with a median number of 3 0 in group I and in group II 2 0 All children in both groups attended the 2 year follow up which included a case history with special reference to atopic symptoms All sera were stored at −20°C until analyzed

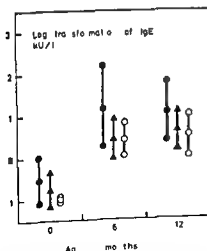


Fig 1 Serum IgE concentrations Children with onset of atopic symptoms during the follow up (●) and asymptomatic children (▲) in Group I compared to controls (O) (Group II)

The mothers were interviewed shortly after delivery by one of us (AD) to elucidate the case and family history with regard to atopic disease During the follow up all children were investigated by the same clinician and the parents were questioned regarding the children s diet but were not consciously influenced by the clinician At each attendance the children were classified as definitely atopic possibly atopic or non atopic The children who did not come for investigation were classified from data obtained from telephone cells

The following definitions were used

Definite atopy evident symptoms of atopic dermatitis allergic urticaria bronchial asthma and intestinal reactions of immediate type which were clearly related to ingestion of a specific food

Possible atopy a convincing history of itching eczema a single episode of urticaria of unknown cause circumoral rash by direct contact with food wheezing during respiratory infections or gastro intestinal disturbances such as prolonged periods of non infectious diarrhoea not shown to be related to the diet

The term food allergy is used for reactions in which an immunologic basis can be demonstrated In other cases the term food intolerance is used

METHODS

IgE concentrations were measured by a direct sandwich type of radioimmunosorbent technique PRIST (3) Quantitative determinations of serum IgA were made by single radial immunodiffusion in agar gel (17)

The IgE antibody activity was determined by the radioallergosorbent test RAST (14) using ^{125}I labelled anti De2 The results were expressed in PRU/ml (Phadebas

Table 2 *Serum IgA concentrations in children of atopic mothers (Group I) and in children of healthy mothers (Group II)*

The difference was statistically significant at 6 months of age

	Group I		Group II	
Age (months)	No of children	IgA g/l	No of children	IgA g/l
0	36	0 017 (0 008–0 018)	17	0 012 (0 009–0 018)
3	9	0 08 (0 05–0 14)	1	0 10
6	8	0 16 (0 11–0 25)	8	0 72 (0 17–0 42)
17	25	0 21 (0 13–0 36)	7	0 24 (0 14–0 40)
18	11	0 30 (0 17–0 50)	1	0 18
24	9	0 31 (0 18–0 54)	1	0 36

The geometric mean the geometric mean ± S D are indicated in parentheses

RAST Units Pharmacia Diagnostics AB Uppsala) and >0 1 PRU/ml was considered positive

IgG antibodies to the same allergens were measured by a method based on the binding of IgG to staphylococcus protein A coated Sepharose (6 13) using ^{125}I labelled allergen

Skin prick tests were performed with a 100 mg/ml solution of the same allergen preparations as were used in the in-vitro assays A wheal size exceeding the histamine control by 2 mm or more was considered positive The concentration of histamine was 1 mg/ml Allergen preparations used were β-lactoglobulin (BLG) (100 mg per hundred paper discs) (Miles Laboratories Ltd lot no 96007-04) and ovomucoid (OM) from raw egg white (60 mg per hundred paper discs) (Miles Laboratories Ltd lot no 96-016)

For statistical comparison of the antibody levels of the different groups Student s *t* test was used

RESULTS

Symptoms

None of the children of the healthy mothers but 50% of the children of the atopic mothers developed atopic symptoms during the 2 years follow up period Eight children were considered as definitely atopic and 10 as possibly atopic according to the criteria given Mean age at onset in the definitely atopic children was 10 months and in the possibly atopic children 13 months The most common manifestations in children considered to be definitely atopic were eczema (six children) urticaria (5 children) and gastrointestinal symptoms (5 children) The most common foods suspected to cause adverse reactions were in order egg tomatoes citrus bananas chocolate milk and fish

The duration of complete breast feeding was similar in both groups and in about 40% of the infants a cow s milk formula was introduced before the age of 3 months All atopic children had complete breast feeding for more than 2 weeks (definitely atopic children mean 5 3 months possibly atopic children mean 3 2 months non atopic children mean 2 5 months) No relationship between the development of allergic symptoms and time for onset of artificial feeding could be demonstrated According to the parents opinion 25% of the children of atopic mothers were prone to upper respiratory infections as compared to 6% of the controls

Table 3 *Serum concentrations of IgG antibodies to β-lactoglobulin (BLG) and ovomucoid (OM) in mothers and newborns*

	IgG antibodies to BLG	IgG antibodies to OM
Group I		
Mothers	10 8 (1 7–68 3)	43 7 (7 4–257)
Newborns	9 3 (7 2–39 0)	47 7 (7 9–625)
Group II		
Mothers	6 9 (1 2–38 4)	76 9 (6 7–108)
Newborns	4 4 (1 0–19 4)	78 8 (4 5–185)

The concentrations are expressed in % of a reference geometric mean the geometric mean ±1 S D are indicated in parentheses

Table 4 *Three children with detectable amounts of IgE antibodies to β-lactoglobulin (BLG)* Number 1 with cow s milk allergy Number 2 and 3 without obvious intolerance to cow s milk

Case no	Age (months)	IgE (kU/l)	IgE BLG (PRU/ml)	IgG BLG (%)	Skin test BLG
1 (FJ)	6	350	0 6	2 800	Positive
	12	290	0 6	9 500	
	24	270	0 4	4 200	
2 (AZ)	[illegible]	1 2	<0 04	5	
	12	54	<0 04	19	
	24	230	0 11	36	Positive
3 (AS)	12	7	0 12	4 300	
	18	6 2	<0 [illegible]	7 000	
	24	9 5	0 08	6 500	Positive

ᵃ PRU Phadebas RAST Units

IgE levels

The IgE levels were significantly ($p<0\ 01$) higher in the atopic mothers (geom mean 60 1 range 5 4–500 kU/l) than in the healthy mothers (geom mean 18 2 range 4 3–83 kU/l) The mean IgE level in the cord sera was significantly ($p<0\ 01$) higher in group I (geom mean 0 24 kU/l) than in group II (geom mean 0 11 kU/l) No correlation was found between the maternal serum and the cord serum IgE levels The IgE concentrations in group I were higher during the whole follow up period (Table 1)

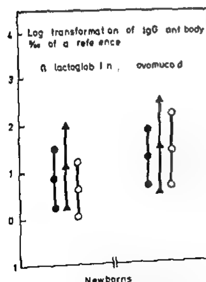


Fig 2 The cord serum concentration of IgG antibodies to β lactoglobulin and ovomucoid Children with onset of atopic symptoms during the follow up (●) and asymptomatic children (▲) in Group I compared to controls (O) (Group II)

An elevated cord serum IgE level seemed to be predictive of later onset of atopic symptoms (Fig 1) In this study all five children with a cord serum IgE level exceeding 0 4 kU/l developed atopic symptoms during the observation period In children who developed atopic symptoms the serum IgE levels were significantly higher ($p<0\ 05$) at 6 and 12 months of age than in the controls and in the other children of group I who remained asymptomatic At 3 18 and 24 months the number of samples was too small for statistical analysis

IgA levels

The cord IgA levels were low and similar in both groups and bore no relationship to atopic heredity or to later development of atopic symptoms The IgA levels were lower in group I than in group II when the infants were 6 months of age ($p<0\ 1$) Children with atopic symptoms had higher levels although not statistically significant as compared with the asymptomatic children The IgA levels were similar in all children in group I and II at 12 months of age (Table 2) In the whole series all serum IgA levels were within normal limits for the age

IgG and IgE antibodies to β-lactoglobulin and ovomucoid

IgG antibodies to BLG from cow s milk and ovomucoid from egg white were detected in

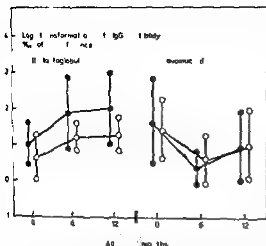


Fig 3 The serum concentration of IgG antibodies to β lactoglobulin and ovomucoid in children of atopic mothers (●) (Group I) and in children of healthy mothers (O) (Group II) The concentrations expressed in % of a reference the geometric mean and the geometric mean ±1 S D

most maternal sera The titers were higher in the atopic mothers than in the controls (Table 3) IgE antibodies to BLG and OM were found in only one mother in low titers In addition this mother had high titers of IgG antibodies to these allergens She had asthma and rhinitis on animal exposure but no symptoms of milk or egg allergy A skin prick test was positive with OM but negative with BLG

In cord sera no IgE antibodies to BLG or OM were detected

The cord serum levels of IgG antibodies to both allergens were very similar to those of the respective mothers indicative of a transport across the placental barrier Among group I children those who remained asymptomatic had higher levels than those who developed atopic manifestations possibly indicating a protective function of the transferred antibodies However this difference was not statistically significant (Fig 2) The primary high BLG IgG antibody levels decreased in most children during the first three months of life and thereafter showed a slow rise (Fig 3) At 6 and 12 months of age the children of group I had significantly ($p<0.05$) higher BLG IgG antibody titres than the controls The primary decrease of the ovomucoid IgG antibody levels was still more pronounced and the levels began to rise first after 6 months of age in most children No effect on the titers by the time of onset of artificial feeding could be demonstrated

IgE and IgG antibodies to BLG in relation to cow s milk intolerance

Cow s milk intolerance was suspected and later verified by challenge in 3 children Two of them had more delayed onset of symptoms from the gastrointestinal tract and no detectable IgE antibodies to BLG In these 2 cases further investigations showed a lactose intolerance rather than an intolerance to the cow s milk protein

The third child had at the age of 4 months immediate onset of diarrhea after cow s milk intake and urticaria when milk was applied to the skin At 6 months of age an elevated serum IgE level and IgE antibodies to BLG were detected and skin prick test with BLG was positive In addition this child (no 1 in Table 4) had a high level of IgG antibodies to BLG The allergy proved to be transient and the child tolerated small amounts of cow s milk already at 12 months of age

Another 2 children (nos 2 and 3) also had detectable amounts of IgE antibodies to BLG but without any obvious allergy to cow s milk One of them (no 2) also had a high titer of IgG antibodies to BLG Both showed weak but positive reactions at skin prick testing with BLG at 24 months of age One child had a moderate eczema (no 2) and the other (no 3) had a history of recurrent otitis

IgE and IgG antibodies to ovomucoid in relation to egg intolerance

According to the parents 4 children in group I did not tolerate eggs One of them had detectable IgE antibodies to OM at 24 months of age This child also had a high titer of IgG antibodies to OM The onset of symptoms as urti

caria and eruption of eczema when eating egg white containing food preceded the occurrence of IgE antibodies with 6 months Skin prick test with OM performed at 24 months of age was positive in this child and in one of the other 3 children Another 6 children tolerant to eggs including one child in the control group also had IgE antibodies to ovomucoid in even higher levels than the infants with clinical symptoms of egg intolerance Three of them had such symptoms as asthmatoid bronchitis, eczema and prolonged diarrhea but none was improved after egg elimination Skin prick tests with OM at the age of 24 months were positive in 4 of the 6 children The OM IgG antibody titers paralleled the corresponding IgE antibody titers and the mean titer (336%) at 12 months age was significantly higher than the mean titer (14 2%) of the other children in group I at the same age ($p<0\ 05$)

DISCUSSION

The elevated serum IgE levels and the case history of atopic allergy in the mothers of group I indicate that their children could be considered as predisposed to atopic disease and suitable for a study of the immune response in relation to the development of atopic symptoms (15) This showed to be true as 50% of these children developed atopic symptoms during the observation time as compared with none of the controls

In agreement with earlier reports there was no correlation between IgE in the mothers and their newborns indicating that IgE in cord serum is of foetal origin This study confirms that elevated IgE in cord serum is predictive of subsequent development of atopic symptoms (9, 17, 19)

IgE antibodies to BLG and ovomucoid were not detected in cord sera but several children in group I developed low titers In addition one of the children in the control group had IgE antibodies to ovomucoid The correlation between the occurrence of these antibodies and clinical symptoms was found to be low in this study as in others (4 5 22)

Secretory IgA is reported to have an important role in immune exclusion of antigens in the gut and a deficient or immature IgA system could possibly predispose for allergy (24) In our study this is seemingly supported by the fact that low serum IgA concentrations were found in the group I children at the age of 6 months However this was the case only in group I children who were symptom free during the follow up period In our experience and in that of others atopic children who develop IgE antibodies also have an elevation of the serum IgA antibody levels (15)

Children of atopic mothers often had high levels of transferred maternal IgG antibodies to BLG and OM These antibodies are possibly able to contribute to antigen elimination Symptom free children in group I had higher titers than those who developed allergic symptoms which indicates a possible protective function of these antibodies

High IgG antibody titers to foods are often seen in children who are developing tolerance to the same food and in our experience these titers are not strictly correlated to food antigen intake The IgG food antibody titers are often high even in children who have avoided the appropriate food for a long time In accordance with an earlier study we found that the IgE and IgG antibody titers to BLG and OM seemed to parallel each other (4) However the occurrence of IgE antibodies preceded that of IgG antibodies in some cases The clinical significance of IgE antibodies to milk and egg white has been disputed and therefore challenge tests are considered necessary in dubious cases

In infancy even low titers of IgE antibodies to food seem to be able to give anaphylactic reactions while on the other hand high titers can be consistent with tolerance This can possibly be explained by the fact that the massive antigenic load on the gut associated lymphoid tissues favours not only formation of IgE antibodies but also a rapid development of

IgG IgA and IgM food antibodies with [redacted] possible blocking capacity. The gut permeability and factors regulating the local sensitivity of the target organs will also influence the effects of IgE antibodies to foods In conclusion in vitro tests are at present considered to be of limited value in the diagnosis of food allergy in infancy However the occurrence of an elevated IgE level in relation to age and IgE antibodies to milk and egg can indicate an atopic disposition and a possible component of reaginic food allergy In such cases an elimination diet and subsequent challenge with the appropriate food can sometimes confirm the diagnosis of food allergy

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EFFECTS OF STABILIZERS IN PREPARATIONS OF HUMAN SERUM ALBUMIN ON THE SERUM BILIRUBIN IN GUNN RATS

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From Kaiserin Auguste Victoria Haus Children s Hospital Free University Berlin Germany

ABSTRACT Ballowitz L, Schmid J Siegert M and Steffen B (Kaiserin Auguste Victoria Haus Children s Hospital Free University Berlin Germany) Effects of stabilizers in preparations of human serum albumin on the serum bilirubin in Gunn rats Acta Paediatr Scand 67 505 1978.—Commercially available preparations of human serum albumin (HSA) containing stabilizers (i e 16 mmol/l Na caprylate plus 16 mmol/l Na N acetyl DL-tryptophan) were injected either s c i p or i v into homozygous infant Gunn rats 30 min and 3 hours after s c injection a serum bilirubin decline which surpassed dilution by the injected volume could be ascertained It was mainly caused by N acetyl DL-tryptophan since s c injections of appropriate amounts of this substance alone or a mixture of both components of the stabilizer without HSA brought about similar results HSA without these stabilizers had not such an effect It is postulated that under these conditions Na N acetyl DL tryptophanate displaced bilirubin from albumin bonds It became obvious that after s c injection equilibration of HSA between skin and plasma was delayed whereas Na N acetyl DL-tryptophan was rapidly transported into the blood As for Na caprylate a displacing effect of short duration could not be excluded by the experimental arrangement used since the metabolism of the substance in the rat is very fast When HSA and the stabilizers entered the plasma simultaneously (i v injection) no effect on serum bilirubin concentration could be proved 30 min and 3 hours later All the bilirubin and the Na N acetyl DL-tryptophan present in the plasma at that time can be bound to the large amount of albumin which is directly given into the circulation of the animal 30 min after i p injection of HSA preparations containing stabilizers a small decrease of serum bilirubin concentration could be recognized It was less pronounced and less persisting than after s c injection Probably equilibration of HSA between peritoneum and plasma went on faster than between skin and plasma Only for a short period a lack of albumin binding sites in the plasma of the rat pointed to a surplus of Na N acetyl DL-tryptophan

KEY WORDS Displacement of bilirubin albumin bonds kernicterus Gunn rats

During studies in infant Gunn rats on possible effects of intravenous nutrients (i e lipid emulsions aminoacid solutions and different plasma protein preparations) on bilirubin albumin binding we found hints for bilirubin displacement by commercial preparations of human serum albumin (HSA) Recently Brodersen & Hansen (4) reported on displacing effects on stabilizers in injectable preparations of HSA The stabilizers are added to protect the protein during heat treatment to prevent transmission of hepatitis For in vitro tests the Danish authors used the peroxydase method Taking their quantitive findings as a basis they roughly estimated the effect of these additives on the concentration of the free (unbound) bilirubin in blood plasma after HSA infusion in permitted amounts They suspected a delay of the desired decrease of free bilirubin concentration or even a temporary increase induced by the stabilizer after HSA infusions into icteric neonates In the Gunn rats the effect on total serum bilirubin varied with different application

MATERIAL AND METHODS

We primarily injected 3–5-day-old homozygous Gunn rats subcutaneously (s c) with 0 02–0 08 ml/g of various com

man A F & Taylor B Predisposing factors and the development of reaginic allergy in infancy *Clin Allergy* 6 305 1976
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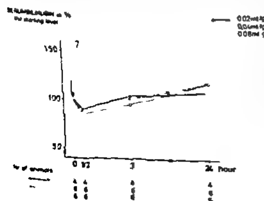
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Table 1 *Mean serum bilirubin in % of the starting level in infant Gunn rats after injection of several HSA preparations and stabilizers (STAB) (investigation series 2)*

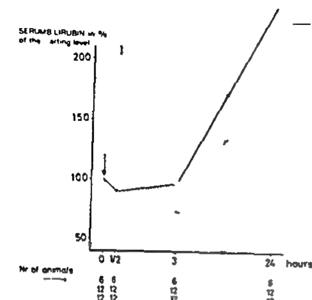
	Time after injection			
	30 min	3 h	24 h	48 h
s c 0 08 ml/g				
20% HSA with STAB = 16 mmol/l Na caprylate + 16 mmol/l Na N acetyl-DL tryptophan	62	60	189	286
20% HSA without STAB	93	87	165	303
5% HSA with STAB = 4 mmol/l Na caprylate + 4 mmol/l Na N acetyl DL tryptophan	59	63	160	211
STAB mixture (P 7 0) = 16 mmol/l Na caprylate + 16 mmol/l Na N acetyl DL tryptophan	59	75	113	
Na caprylate 16 mmol/l (P_H 6 8)	83	106	117	120
Na N acetyl DL tryptophan (P_H 4 9) 16 mmol/l	60	82	115	110
0 9% NaCl	86	92	109	108
i p 0.04 ml/g				
20% HSA with STAB	90	101	187	
20% HSA without STAB	109	118	199	270
i v 0 02 ml/g				
20% HSA with STAB	170	157	199	250
20% HSA without STAB	161	143	207	219
STAB mixture	80	92	114	
Na caprylate	97	117	117	
Na N acetyl DL tryptophan	79	83	109	
0 9% NaCl	91	106	119	131

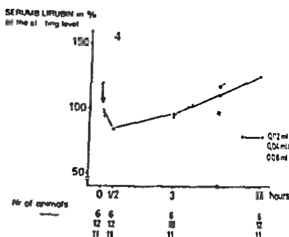
mercially available HSA preparations and appropriate amounts of stabilizers (i e Na caprylate 16 mmol/l and Na N acetyl DL tryptophan 16 mmol/l) Control animals received equal volumes of a 0 9% NaCl solution Moreover the manufacturers i e Behringwerke and Biotest supplied us with HSA without stabilizers In a second series some of the preparations were administered to infant Gunn rats intraperitoneally (i p) or intravenously (i v) and finally some 1 month-old rats were similarly tested Blood was taken from the cut tail and serum bilirubin concentration directly read on the American Optical Bilirubinometer Serum protein was estimated on biuret basis with centrifichem autoanalyzer (Roche) and with agarose gel-electrophoresis (see Siegert & Siemens (8))

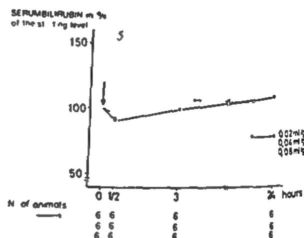


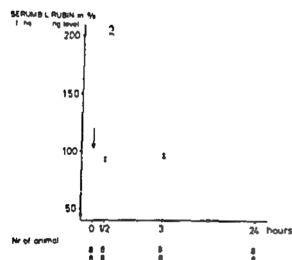
RESULTS

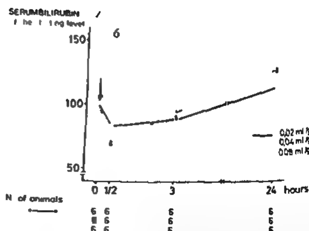
In Figs 1–7 serum bilirubin curves can be compared These curves are taken from the thesis of B Steffen (9) All the preparations which contain Na N acetyl DL tryptophan or both stabilizers induce a marked dose related serum bilirubin decline after s c injection In Table 1 the results of the 2nd series are given in numbers In this 2nd series serum protein was measured beside serum bilirubin As in series 1 s c injection of all preparations containing Na N acetyl DL tryptophan was followed by a serum bilirubin decline down to about 60% of the starting level after 30 min Because of the small number of animals in each group of series 1 and 2 standard deviations are not mentioned in Figs 1–7 and Table 1 When both series were taken together—after s c injection of 0 08 ml/g—the difference

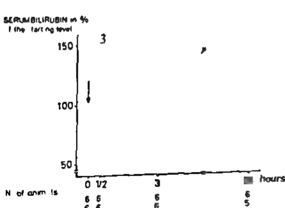










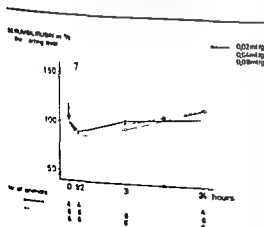


Figs 1–7 Mean serum bilirubin in infant Gunn rats (series 1) after s c injections of 1) 20% HSA with stabilizer (STAB) 2) 20% HSA without STAB 3) 5% HSA with STAB 4) STAB mixture 5) Na caprylate 6) Na N acetyl DL tryptophan 7) 0 9% NaCl

Table 1 *Mean serum bilirubin in % of the starting level in infant Gunn rats after injection of several HSA preparations and stabilizers (STAB) (investigation series 2)*

	Time after injection			
	30 min	3 h	24 h	48 h
s c 0 08 ml/g				
20% HSA with STAB=16 mmol/l Na caprylate+16 mmol/l Na N acetyl DL tryptophan	62	60	189	286
20% HSA without STAB	93	87	165	303
5% HSA with STAB=4 mmol/l Na caprylate+4 mmol/l Na N acetyl DL tryptophan	59	63	160	211
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20% HSA with STAB	90	101	187	
20% HSA without STAB	109	118	199	270
i v 0 02 ml/g				
20% HSA with STAB	170	157	199	250
20% HSA without STAB	161	143	207	219
STAB mixture	80	92	114	
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0 9% NaCl	91	106	119	131

mercially available HSA preparations and appropriate amounts of stabilizers (i e Na caprylate 16 mmol/l and Na N acetyl DL tryptophan 16 mmol/l) Control animals received equal volumes of a 0 9% NaCl solution Moreover the manufacturers i e Behringwerke and Biotest supplied us with HSA without stabilizers In a second series some of the preparations were administered to infant Gunn rats intraperitoneally (i p) or intravenously (i v) and finally some 1 month-old rats were similarly tested Blood was taken from the cut tail and serum bilirubin concentration directly read on the American Optical Bilirubinometer Serum protein was estimated on biuret basis with centrifichem autoanalyzer (Roche) and with agarose gel-electrophoresis (see Siegert & Siemens (8))



RESULTS

In Figs 1–7 serum bilirubin curves can be compared These curves are taken from the thesis of D Steffen (9) All the preparations which contain Na N acetyl DL tryptophan or both stabilizers induce a marked dose related serum bilirubin decline after s c injection In Table 1 the results of the 2nd series are given in numbers In this 2nd series serum protein was measured beside serum bilirubin As in series 1 s c injection of all preparations containing Na N acetyl DL tryptophan was followed by a serum bilirubin decline down to about 60% of the starting level after 30 min Because of the small number of animals in each group of series 1 and 2 standard deviations are not mentioned in Figs 1–7 and Table 1 When both series were taken together—after s c injection of 0 08 ml/g—the difference

Table 2 *Mean serum bilirubin in infant Gunn rats after s c injection of 0 08 ml/g HSA and stabilizers (STAB) Results of series 1 and 2 combined*

	Prior to injection	Time after injection 30 min	3 h	24 h	48 h
20% HSA with STAB serum bilirubin in μmol/l	135±24	84±15	84±20 5	236±39	352±34
In % of the starting level	100%	64%	62%	179%	287%
No of animals	45	29	40	33	8
20% HSA without STAB serum bilirubin in μmol/l	125±20 5	120±26	113±22	229±37 5	–
In % of the starting level	100%	95%	90%	184%	–
No of animals	17	12	17	17	–
Na caprylate 16 mmol/l serum bilirubin in μmol/l	145±29	126 5±31	154±38	169±34	164±14
In % of the starting level	100%	87%	106%	116%	121%
No of animals	25	21	24	24	4
Na N acetyl DL tryptophan 16 mmol/l serum bilirubin in μmol/l	154±36	96±27	126 5±29	178±34	171±34
In % of the starting level	100%	62%	82%	116%	111%
No of animals	30	26	29	30	4

between 20% HSA with and without stabilizer on the one hand (Wilcoxon U test $p_2<0.01\%$) and between Na caprylate and Na N acetyl DL tryptophan on the other hand (Wilcoxon U test $p_2<1\%$) proved statistically significant

Table 2 gives the serum bilirubin concentration in μmol/l with standard deviations for these 4 groups The T test showed a significant distinction between the baseline concentration and the bilirubin concentration 30 min after s c injection of 20% HSA with stabilizer ($p<0.01$) and after injection of Na N acetyl DL-tryptophan ($p<0.01$)

In the curves and the tables serum bilirubin concentration prior to injection was indexed at 100 and the following measurements are given in percentages of this baseline concentration (being between 120–170 μmol/l) There is practically no serum bilirubin decline after injection of saline of Na caprylate and of HSA *without* stabilizer Whereas Na N acetyl DL tryptophan a mixture of both stabilizers and 20% HSA as well as 5% HSA *with* stabilizer provoked a clear decrease *30 min and 3 hours after s c injection* A comparison of the curves suggests that not the HSA per se but the stabilizer had induced this decrease and that Na N acetyl DL tryptophan was mainl responsible for the effect

24 hours after s c injection of preparation not containing HSA the total serum protei concentration of the animals had practicall not changed the starting level of serum bil rubin was slightly surpassed This correspond to the physiologic serum bilirubin increase i Gunn rats of this age (≈8 5 μmol/l per day) 24 hours after injection of HSA a sharp serur bilirubin increase occurred It seemed likely that an excessive binding capacity in the plas ma had now been reached causing bilirubi to penetrate from the tissues to plasma albu min

After s c injection of 0 08 ml/g 20% HS the total serum protein of the rats distinctly ir creased by 13 0–16 0 g/l at 3 hours and abou 20 g/l at 24 hours after the injection (base line concentrations being 27–35 g/l) With th electrophoresis the human albumin peak wa clearly distinguishable from the albumin pea of the rat Human albumin moved faster tha the rat albumin (Fig 8) 3 hours after s c in jection of 0 08 ml/g 5% HSA total serum pro tein increased by about 4 0 g/l and at 24 hour by about 10 0 g/l

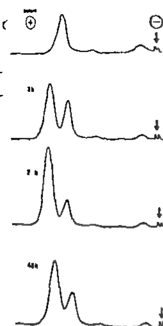


Fig 8 Densitometer curves of agarose gel electrophoresis of infant Gunn rat serum before and after s c injection of 0 08 ml/g 20% HSA with stabilizer (aminoblack 587 nm E 2 0 barbital HCl buffer P_H 8 4 ↓=point of application)

	Albumin in g/l	in % of total serum protein
Before injection		
Rat	? 9	61
3 h after injection		
Human	30 5	57
Rat	16 0	30
24 h after injection		
Human	40 0	70
Rat	11 5	?0
48 h after injection		
Human	34 4	58
Rat	17 ?	?9

Beside the Behringwerke products (Figs 1-3) we tested the commercially available 20% HSA produced by Biotest which contains similar stabilizers (Na caprylate 17 6 mmol/l plus Na N acetyl tryptophan 17 3 mmol/l) and two special preparations made available by Biotest for these investigations namely (*a*) 16 9% HSA with stabilizer (Na caprylate 14 8 mmol/l plus Na N acetyltryptophan 14 6 mmol/l) and (*b*) 21 6% HSA of the same plasma pool without stabilizer the latter being subjected to β propiolactone+uv irradiation in order to inactivate hepatitis virus (Lo Grippo (7))

The results obtained after s c injection with the Biotest products with respect to serum protein increase and influence on serum bilirubin concentration were in the same range as results obtained with the Behringwerke products No special influence of the LoGrippo procedure could be ascertained

The Behringwerke products and the stabilizers were also injected i p and i v Already 3 hours after 0 04 ml/g 20% HSA i p serum protein increased by 16-18 g/l and nearly the same concentration was found at 24 hours 30 min and 3 hours after i p injection of 20% HSA with stabilizer serum bilirubin was at 90% and 101% respectively and at 109% and 118% when 20% HSA without stabilizer was injected (Table I) A small decrease by the stabilizer could be recognized

After 0 02 ml 20% HSA i v (the highest dose tolerated by the infant rats) an increase of serum protein of about 10 0 g/l could be noticed 30 min and 24 hours later At 3 hours a passing decline was obvious—probably induced by osmotic hypervolemia Serum bilirubin concentration sharply increased after i v injection of 20% HSA to about 160-170% of the starting level after 30 min and to about 200-210% after 24 hours 3 hours after the injection we measured 143-157% With i v application no clear difference could be ascertained between preparations with or without stabilizers

Bilirubin decrease after i v injections of the *stabilizers*—0 02 ml/g—was practically identical to that following s c application of the same dose i e no decrease after Na caprylate but a decrease to 80% after Na N acetyl DL tryptophan

DISCUSSION

Brodersen (3) found in 1974 that N acetyl tryptophan displaces bilirubin in vitro from its complex with serum albumin Bourgoin and coworkers (2) recently demonstrated a de

creased number of available binding sites for tryptophan on the (defatted) serum albumin of infant rats during the first week after birth compared to adult rats However, the apparent association constant of tryptophan binding to serum albumin was similar in newborn and adult animals Distinct differences on serum albumin of newborns and adults at the association site for tryptophan are suspected to induce the alteration in the binding capacity on the protein

These observations of Bourgoin et al induced us to repeat some of our investigations in 1 month old rats Baseline concentration of serum bilirubin was lower and the degree of displacement from albumin binding sites by the stabilizers was smaller than in 3–5 day old rats We found no evidence for more intense Na N acetyl DL tryptophan binding to albumin *in the older animals* The general findings agreed no remarkable difference between HSA with and without stabilizer after i v application bilirubin displacement by Na N acetyl DL tryptophan after i p as well as after s c injection displacement after s c administration of HSA *with* stabilizer

The different results after s c (and i p) injections on the one hand and i v on the other hand can only be explained by assuming that equilibration of HSA and stabilizer (at least in the case of Na N acetyl DL tryptophan) following s c (or i p) application is quite different From our serum protein measurements we know that after s c injection penetration of HSA into the circulation will be delayed and probably not totally finished within 24 hours (Dewey (5) reported that in the rat equilibrium of ^{131}J labelled albumin between plasma and tissues such as fat skin and muscle required up to 3 days) The distribution of Na N acetyl DL tryptophan or Na caprylate was not experimentally proved by us but a rapid tissue/blood transport of amino acids—i e Na N acetyl-DL tryptophan—and of medium chain fatty acids—i e Na caprylate—can be assumed It is very likely that after s c injection of commercially available HSA preparations a high Na N acetyl DL tryptophan concentration in plasma is reached within 30 min and that the substance will be metabolized (eliminated from plasma) within 24 hours In infant rats metabolism of capric acid is rather fast (see Warshaw (11)) In case the 460 μg Na caprylate injected with 0 2 ml 20% HSA into a 10 0 g rat are the only caloric supply they will be metabolized in about 1 min

We may thus conclude that in the animal model used by us the bilirubin displacing effect of Na N acetyl DL tryptophan added to injectable HSA had a distinct effect on serum bilirubin only because the distribution of HSA and stabilizer was different in the body of the rat after s c injection When both ingredients entered the plasma simultaneously (i v) we were unable to prove an effect in the jaundiced homozygous Gunn rat *If injected* together i v , the amount of Na N acetyl DL tryptophan and bilirubin present at 30 min can be bound to the large amount of albumin Subcutaneous injections of HSA are clinically irrelevant Fette (6) investigated (in our hospital) serum bilirubin and serum albumin in newborn babies after i v injection of 5% and 20% commercial HSA (with stabilizer) His curves show the same tendency as the animal tests

As for Na N acetyl DL tryptophan our observations largely agree with the results and calculations by Brodersen & Hansen (4) They stated that in an icteric infant an i v dose of 5 ml/kg 20% HSA+40 mmol/l Na N acetyl tryptophan administered together is likely to influence free bilirubin concentration in plasma in the following range 2 fold increase/decreased to half of the level prior to injection —varying with the concentrations of bilirubin and albumin before treatment —Obviously the drug induced bilirubin decline in the icteric Gunn rat is reciprocally in agreement with the amount of displaced bilirubin demonstrable as free bilirubin in the plasma in vitro

As regards Na caprylate Brodersen & Hansen (4) found a markedly more pronounced displacing effect by the peroxydase meth

od compared to Na N acetyl DL tryptophan They calculated an in vivo displacement of the same order and magnitude as that of Na N acetyl DL tryptophan According to Ashbrook et al (1) and Thiessen et al (10) binding of caprylate is largely influenced by long chain fatty acids The content of lipids and fatty acids in the plasma of infant Gunn rats is high (also in the milk of rats) We have not yet been able to prove or to exclude as to whether the interaction of fatty acids or a rather fast elimination of caprylate will be responsible for the ineffectiveness of Na caprylate in vivo But it seems most likely that the Na caprylate was already metabolized 30 min after the injection the time at which our first tests were done

Basic metabolic rate of a human neonate is relatively smaller than that of an infant Gunn rat It can therefore not be excluded that a certain amount of Na caprylate may remain long enough in the blood plasma of a human neonate to effect a delay of bilirubin extraction from the tissues—or possibly even a temporary displacement of bilirubin from plasma albumin A similar conclusion can be made for Na N acetyl DL tryptophan

The slight differences between in vitro and in vivo (Gunn rat) observations support the statement of Yeary & Davis (12) that a completion of in vitro results on bilirubin displacement by drugs by the animal test is desirable The animal model provides a basis for establishing deviations as a result of drug dosage the time course of drug action the possible role of metabolites and the influence of additional metabolic processes

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creased number of available binding sites for tryptophan on the (defatted) serum albumin of infant rats during the first week after birth compared to adult rats However the apparent association constant of tryptophan binding to serum albumin was similar in newborn and adult animals Distinct differences on serum albumin of newborns and adults at the association site for tryptophan are suspected to induce the alteration in the binding capacity on the protein

These observations of Bourgoin et al induced us to repeat some of our investigations in 1 month old rats Baseline concentration of serum bilirubin was lower and the degree of displacement from albumin binding sites by the stabilizers was smaller than in 3–5 day old rats We found no evidence for more intense Na N acetyl DL tryptophan binding to albumin in the older animals The general findings agreed no remarkable difference between HSA with and without stabilizer after i v application bilirubin displacement by Na N acetyl DL tryptophan after i p as well as after s c injection displacement after s c administration of HSA *with* stabilizer

The different results after s c (and i p) injections on the one hand and i v on the other hand can only be explained by assuming that equilibration of HSA and stabilizer (at least in the case of Na N acetyl DL tryptophan) following s c (or i p) application is quite different From our serum protein measurements we know that after s c injection penetration of HSA into the circulation will be delayed and probably not totally finished within 24 hours (Dewey (5) reported that in the rat equilibrium of ¹³¹J labelled albumin between plasma and tissues such as fat skin and muscle required up to 3 days) The distribution of Na N acetyl DL tryptophan or Na caprylate was not experimentally proved by us but a rapid tissue/blood transport of amino acids—i e Na N acetyl DL tryptophan—and of medium chain fatty acids—i e Na caprylate—can be assumed It is very likely that after s c injection of commercially available HSA preparations a high Na N acetyl DL tryptophan concentration in plasma is reached within 30 min and that the substance will be metabolized (eliminated from plasma) within 24 hours In infant rats metabolism of capric acid is rather fast (see Warshaw (11)) In case the 460 µg Na caprylate injected with 0 2 ml 20% HSA into a 10 0 g rat are the only caloric supply they will be metabolized in about 1 min

We may thus conclude that in the animal model used by us the bilirubin displacing effect of Na N acetyl DL tryptophan added to injectable HSA had a distinct effect on serum bilirubin only because the distribution of HSA and stabilizer was different in the body of the rat after s c injection When both ingredients entered the plasma simultaneously (i v) we were unable to prove an effect in the jaundiced homozygous Gunn rat If injected together i v the amount of Na N acetyl DL tryptophan and bilirubin present at 30 min can be bound to the large amount of albumin Subcutaneous injections of HSA are clinically irrelevant Fette (6) investigated (in our hospital) serum bilirubin and serum albumin in newborn babies after i v injection of 5% and 20% commercial HSA (with stabilizer) His curves show the same tendency as the animal tests

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TRANSIENT ERYTHROBLASTOPENIA IN CHILDHOOD

A Study of 15 Cases

RUTH WEGELIUS and THEODOR H WEBER

From the Aurora Hospital Department of Paediatrics and Clinical Laboratory Helsinki Finland

ABSTRACT Wegelius R and Weber T H (Department of Paediatrics and Clinical Laboratory Aurora Hospital Helsinki Finland) Transient erythroblastopenia of childhood A study of 15 cases Acta Paediatr Scand 67 513 1978 —A survey is presented of 15 patients from the Aurora Hospital and 35 patients reported in the literature with transient erythroblastopenia of childhood (TEC) The children were hospitalized because of pallor and tiredness some of them having signs of mild infection They displayed normochromic anaemia reticulocytopenia and erythroblastopenia during the severe stage of the disease In addition they had moderately high values for serum iron and iron binding saturation No other haematological chemical or cytogenetic abnormalities could be demonstrated 80% of the children were between 6 and 48 months old and the sexes were equally affected In the 15 patients from the Aurora Hospital a barely significant (p=0 02–0 05) association with blood group A was recorded Remission indicated by a rise in the reticulocyte count begins within a week after the diagnosis is made even without treatment The aetiology of the disease is unknown Analysis of the red blood cell population at the time of diagnosis indicates that damage to the red cell precursors has occurred 2–3 months before the child is brought for examination

KEY WORDS Erythroblastopenia normochromic anaemia hypoplastic anaemia

Transient erythroblastopenia of childhood (TEC) is characterized by normocytic normochromic anaemia with severe reticulocytopenia and erythroblastopenia Leuco- and thrombopoiesis remain undisturbed The serum iron values are at or above the upper limit of normal The disease is self limiting Relapses have not been shown to occur The disorder has been found only in infants and young children and has not been reported in adults The aetiology is unknown

Similar conditions have been described previously by Lovric in 12 patients Wranne in 4 patients Wang & Meintzer in 9 patients Prindull et al in 5 patients Tenstam in 3 patients and Ritter & Zeller in 2 patients (8 12 13 14 15 16) Since 1964 15 patients with this disease have been seen at the Aurora Hospital Helsinki

MATERIALS AND METHODS

Our series comprised 15 consecutive cases in 8 boys and 7 girls all Caucasians admitted to the hospital between 1964 and 1976 At the time of diagnosis the youngest patient was aged 1 month and the oldest 4 years and 4 months The age distribution is shown in Fig 1 Nine patients gave a history of some mild infection laryngitis gastroenteritis or a febrile disease 1 to 3 months *before* the anaemia was diagnosed One child had viral meningitis on admission In all cases the reason for examination of the child was gradually increasing tiredness pallor lack of appetite and in some cases a low haemoglobin value *found incidentally* at a routine examination These were also the only clinical findings on admission to hospital except in the child with meningitis The various laboratory tests were performed by standard methods The erythroid/myeloid ratio in bone marrow was evaluated by

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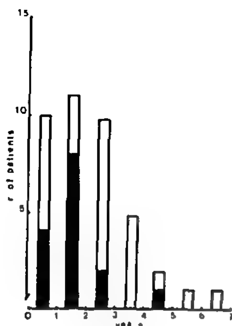


Fig 1 Age distribution of patients with TEC in this investigation (black bars) and from literature (open bars) (8 12 13 14 16)

counting 400 cells The search for erythroblast antibodies in the serum was performed by indirect immunofluorescence according to Linder s method (7)

RESULTS

Laboratory data (Table 1) revealed normocytic/normochromic anaemia with reticulocytopenia The white blood cell and platelet counts were normal All children showed a slight predominance of mononuclear cells over granulocytes in the peripheral blood In the bone marrow the erythroid/myeloid ratio was decreased but the erythroblasts were morphologically normal Myelopoiesis and thrombopoiesis were normal In case 15 slight megaloblastoid changes were seen

Serum iron was normal or slightly elevated with normal iron binding capacity and high iron binding saturation In 3 children cases 11 14 and 15 the erythropoiesis had begun before admission to hospital as indicated by the increased reticulocyte count The lower serum iron values in these children probably reflect the increased demand for iron during recovery or a low basal value common in children of that age Inclusion in the series was based on development of symptoms laboratory data and the course of the disease during the time of observation In case 15 the child was born at full term after an uncomplicated pregnancy The Hb value of the mother was 13 g/dl The postnatal period was uneventful The child s low Hb value was found at routine examination The mother then recalled that the baby might have been pale during the first few days but he was not considered to be ill No foeto-maternal or other haemorrhage nor haemolytic anaemia nor any intrauterine or postnatally acquired disease could be demonstrated Haemolytic and other anaemia could also be excluded in cases 11 and 14 Of the 15 children 11 belonged to blood group A and 14 were rhesus positive In all patients Coomb s test for erythrocyte antibodies was negative Serum vitamin B_{12} was determined in 6 patients and folate in 7 patients with normal results Foetal haemoglobin was measured in 5 patients and only one case 1 had a slight elevation to 7 5% In 8 patients RBC osmotic fragility was tested and in 4 patients red cell enzymes (glucose-6-phosphate dehydrogenase pyruvate kinase and glutathione reductase) were measured with no abnormal findings Chromosomes of bone marrow cells were analysed in 4 patients all had normal karyotypes A search for erythroblast antibodies in 5 patients gave negative results

Four of the first children of the series were treated with prednisone and 10 received one blood transfusion each All children recovered The time lapse between diagnosis and the onset of reticulocytosis was 0 to 16 days with the exception of one child who recovered after 31 days and was treated with prednisone for 7 days before reticulocytes appeared in the blood Recovery included well being in addition to correction of the Hb value and the other laboratory findings except that in one child serum iron remained below normal after recovery the reason presumably being either an increased requirement or a low basal value in this child The low level was corrected with iron therapy Control bone marrow specimens

Table 1 *Laboratory data on admission to hospital*

Ret = Reticulocytes E/M ratio = erythroid/myeloid ratio in bone marrow Fe sat = Iron binding saturation ND = not determined The lower limit for haemoglobin in Helsinki is for children 2–12 months old 100 g/l and 12–48 months old 106 g/l The normal ranges for MCH and MCV in children over 3 months old are 25–30 pg and 75–85 fl respectively The normal marrow erythroid myeloid ratio is taken as 0 2–1 0 The normal ranges for serum iron are 9–27 μmol/l iron binding capacity 45–70 μmol/l and saturation of iron binding 20–55% The findings in cases 11 14 and 15 are discussed in the text

Pat	Sex	Age (mo)	Hb (g/l)	RBC (×10 /l)	MCH (pg)	MCV (fl)	Ret (%)	E/M ratio	Blood gr (ABO Rh)	S–Fe (μmol/l)	TIBC (μmol/l)	Fe sat (%)
1	♂	27	42	1 6	26	83	0 1	0 16	A +	24 5	ND	ND
2	♀	52	72	2 5	29	83	0 1	0 02	A +	29 6	ND	ND
3	♂	17	49	2 3	[illegible]	74	0 0	0 02	A +	29 5	ND	ND
4	♂	15	[illegible]	2 1	24	ND	0 0	0 02	A +	14 8	70 0	21
5	♀	15	56	2 3	25	79	0 1	0 09	O +	21 4	52 4	41
6	♂	[illegible]	60	2 3	26	78	0 0	0 03	A +	27 4	51 0	54
7	♂	24	83	3 0	8	86	0 0	0 00	A +	37 8	48 6	78
8	♀	31	48	1 6	30	82	0 2	0 16	B −	30 0	52 0	58
9	♀	15	76	2 8	27	77	0 1	0 49	B +	31 8	51 3	62
10	♂	14	56	2 4	29	[illegible]	0 0	0 03	A +	20 6	53 2	39
11	♀	17	94	3 3	29	85	4 7	0 08	O +	10 1	46 9	2
12	♂	8	37	1 5	25	72	0 3	0 06	A +	47 0	53 0	[illegible]
13	♀	11	57	2 1	27	78	0 0	0 09	A +	33 7	50 4	67
14	♀	8	78	3 0	26	79	3 4	0 09	A +	9 8	56 9	17
15	♂	1	48	1 5	32	112	11 9	0 80	A +	11 9	47 2	25

obtained from 7 patients were found to be normal By the end of March 1977 no relapses had occurred the longest observation time for a single patient being 12 years and the total observation time for all patients being about 33 years The mean observation time was 3 3 years

DISCUSSION

The gradually developing pallor without other symptoms apart from intercurrent mild infections in some of the children the haematological findings the few clinical symptoms at the time of diagnosis the short duration of the disease after diagnosis and the spontaneous remission are consistent with TEC

In contrast to haemolytic anaemia the low Hb value is parallelled by a low reticulocyte count There can hardly be any question of an aplastic crisis of haemolytic anaemia as there are no findings supporting the presence of inherited or acquired haemolytic anaemia It is easy to rule out deficiencies of vitamin B_{12} or folate The high S–Fe value and high iron binding saturation presumably reflect a decreased requirement due to temporary cessation of red blood cell formation Fig 1 shows the age distribution of our patients and those reported in the literature As can be seen 80% of cases occur before the age of 4 years and none have been reported after the age of 7 In Lovric s series 4 out of 12 children were less than 6 months old and so was one patient in our series none of the other series included such young patients Thus it may be inferred that the vast majority of the patients with TEC are between the ages of 1/2 and 4 years

TEC should be clearly distinguished from chronic or congenital hypoplastic anaemia (1 2 3 15) and from acute erythroblastopenia (4 5) The former is a chronic disease which usually manifests itself within the first 6 months of life Skeletal abnormalities may be present and the disease is sometimes found in siblings It shows no signs of spontaneous remission The erythropoietin level and MCV and fetal haemoglobin values are elevated and so are the levels of several erythrocyte enzymes (15) We did not measure erythropoietin but our patients did not fulfil any of the other criteria although 4 of them were less than one year old

The syndrome of acute erythroblastopenia as defined by Gasser is acute and selective damage to erythropoiesis of short duration (4 5) It develops in response to various disorders such as infections or immunological disturbances or renal failure or after treatment with drugs These patients show an instability of haemopoiesis dyshaemia which causes other disturbances besides pure erythroblastopenia The patient often has a history of allergic manifestations In our patients we found no underlying diseases or intoxications

In the acute erythroblastopenia of Gasser giant proerythroblasts are found in the bone marrow and the arrest of the erythropoietic system is of such a short duration that anaemia does not necessarily develop (4 5) In our patients who were ascertained because of the anaemia no giant proerythroblasts could be demonstrated

Pure red cell aplasia is an acquired disease of adults occurring primarily in middle age It is a chronic disease which sometimes responds to treatment with steroids or immunosuppressive drugs and is easily distinguished from TEC (6)

There is no firm evidence that treatment is beneficial in TEC Wranne (16) treated 3 of his 4 patients and Lovric (8) 5 of his 12 patients with prednisone The nine children described by Wang et al were given no treatment (15) Tenstam treated his 3 patients with folic acid (14) Three of Prindull s 5 patients and Ritter s & Zeller s 2 patients each received one blood transfusion (12 13) Four of the patients in our series were given prednisone but only in one case with a protracted course were there any indications that the treatment was effective This was the child with viral meningitis Ten children were given one blood transfusion

The aetiology and pathogenesis of the syndrome are obscure Two of our patients had a history of recurrent infections and one had viral meningitis at the time of diagnosis Nine of the children in our series had had mild infections mainly respiratory or gastrointestinal before the anaemia was diagnosed Infections are common in children of that age and it seems doubtful whether the mild infections bore any causal relation to the development of TEC in these children who were otherwise in good health

Parents often give drugs against common colds cough and fever quite indiscriminately to children with the slightest symptoms but after 2–3 months they are not always able to recall what drugs if any out of the large number available were given to the child The fact that TEC does not relapse argues against any agent in the surroundings or commonly used drugs as aetiological factors

The loss of erythroblasts or stem cells might be caused by antibodies Sera of patients 9 11 13 14 and 15 were examined for erythroblast antibodies but none were found However the examination was performed when the children had recovered in some cases several months after the actual damage to the red cell precursors by which time the antibodies might well have decreased to undetectable levels Another cause of the cell damage might have been development of immune complexes affecting the erythroblasts or proerythroblasts The mechanism would then be analogous to the reactions in thrombocytopenia caused by viral infection (10) It is not known whether the erythroblast produces the Fc receptor necessary for this reaction

In the 3 cases in which erythropoietin was measured the results were normal which argues against a basic defect in erythropoietin production as a cause of TEC (15 16) Congenital hypoplastic anaemia and also pure red cell aplasia have been ascribed by some authors to the presence of a humoral inhibitor interfering with erythropoietin receptors (9 11) The presence of humoral inhibitors of erythropoiesis has not hitherto been studied in TEC

The distribution of ABO blood groups among the patients is interesting Eleven of the 15 children in our series belonged to blood group A which in Finland has a prevalence of 41 9% The association between TEC and the A blood group is barely significant at the level

of $p=0.02$–0.05 when tested by the χ^2 test No other authors have reported the blood groups of their patients thus this finding can not be confirmed in a larger series

It is intriguing to speculate about the duration of the disease and the time of onset The normal or low normal MCV values indicate that the circulating erythrocytes in TEC belong to a mature population If we assume that red blood cells have a normal half life of 50–60 days this would imply that the damage to the red cell precursors occurred 2–3 months before diagnosis by which time the peripheral blood contains only 1/2 to 1/3 of the normal number of erythrocytes At the time of examination we were unable to elicit any particular information regarding this point

In 4 children the Hb value had been examined 1–2 months before admission In 3 children it was 91–97 g/l and in one child 114 g/l In six children examined 3–12 months before admission the Hb values had been between 110 and 120 g/l

As most of the patients in this and other series recovered soon after the diagnosis was made it may be inferred that the arrest of erythropoiesis lasts for 2–3 months Thus the common infections noted in some of the children shortly before or at admission could hardly have been the primary cause of the disease The gradual development of the anaemia explains the absence of sudden or dramatic symptoms and the fairly good condition of the child despite the often profound anaemia In this respect the disease is different from the acute erythroblastopenia of Gasser (4 5) The symptomatic phase is apparently of short duration and the disease may well be more common than is suggested by the few reports in the literature for probably many cases go undetected and the child recovers before clinically detectable symptoms develop If erythropoiesis has started when the child is examined for the first time the erythroblastopenic phase is easily overlooked

As no patients have been studied in detail during the initial phases of the disease the time periods suggested above are provisional values based on deductions from available data We do not know which stage of erythropoiesis is primarily affected i e whether the damage occurs to the erythroblasts or to some erythroid precursor cell It would be desirable to examine some patients during the very initial stages of TEC but the changes of doing so appear small as detection would involve the demonstration of a decrease in blood reticulocytes and a reduction in bone marrow erythroblasts in children who were otherwise healthy and symptomless

The recognition of TEC is important in order to avoid unnecessary examinations and ineffective treatment

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CASE REPORT

FATAL BCG INFECTION IN AN IMMUNOCOMPETENT GIRL

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ABSTRACT Pedersen F K Engbæk H C Hertz H and Vergmann B (University Clinic of Paediatrics Department G Rigshospitalet and Tuberculosis Department Statens Seruminstitut Copenhagen Denmark) Fatal BCG infection in an immunocompetent girl Acta Paediatr Scand 67 519 1978 —A 6-year-old girl developed progressive symptoms of increased intracranial pressure starting 5 months after BCG vaccination Thirteen months later craniotomy revealed an epitheloid cell granuloma of the arachnoid occluding the foramen of Magendie No tubercle bacilli were found on histological examination Insertion of a Pudenz shunt relieved the symptoms Six months later generalized BCG infection developed and in spite of treatment with ethambutol rifampicin and isoniazid for 10 weeks death occurred during an episode of increased intracranial pressure Mycobacterium BCG could be cultured from several organs The patient showed no obvious evidence of immunodeficiency as judged on the basis of previous disease history particle concentration of granulocytes B and T lymphocytes in peripheral blood concentration of immunoglobulins in serum response of lymphocytes to transformation with mitogens and antigens and histological findings in the thymus and BCG granulomas

KEY WORDS BCG vaccination complication cerebral BCG granuloma generalized BCG infection

After intracutaneous vaccination BCG disseminates via the lymphatics and blood vessels to regional and distant lymph nodes and organs where it multiplies until cellular immunity develops (21) However symptomatic generalized BCG infection is extremely rare (23) and previously published cases known to us have all occurred in individuals in whom immune deficiency is proved or suspected

This report presents a fatal case of cerebral BCG granuloma and generalized BCG infection in a girl with no evidence of immune deficiency

CASE HISTORY

Patient O J a 6-year-old girl was admitted in May 1974 because of symptoms and signs of increased intracranial pressure

Family history was noncontributory Pregnancy and delivery were normal as was the somatic and mental development Her health had been good previously and she had recovered from chickenpox mumps and measles and had never suffered from severe or frequent infections of any kind Smallpox vaccination at the age of 1 year was followed by a normal primary take

In February 1973 the child was vaccinated intradermally in the deltoid region with 0 1 ml of the routine Danish BCG vaccine the local response was normal Five months later (July 1973) she developed headache vomiting and slowly progressive atactic gait and a further 9 months later also double sight When admitted in May 1974 she was found to have increased intracranial pressure as manifested by papillary oedema and widened sutures of the skull Pneumoencephalography and ventriculography were normal but signs of disturbance of liquor resorption were found by investigation with radioactive labelled liquor A Pudenz shunt was inserted after which all symptoms disappeared but because of recurring blockage of the shunt another craniotomy was performed six months later (December 1974) i e 2? months after the BCG vaccination The arachnoid in the posterior fossa was found to be thickened bluish and granulated occluding the foramen of Magendie Microscopical examination of a biopsy from the arachnoid showed a typical epitheloid cell granuloma with giant cells and infiltration of lymphocytes and plasma cells but no necrosis (Fig 1)



Fig. 1 Section of epithelioid cell granuloma from the arachnoid Hematoxylin eosin stain

Ziehl Neelsen staining revealed no acid fast rods The liquor did not yield any growth of tubercle bacilli on culture A new shunt was inserted after which the symptoms disappeared and the patient was discharged from hospital

Three to four months later fatigue varying degrees of fever loss of weight and tendency to skin haemorrhages developed resulting in renewed hospitalization in August 1975 i e 30 months after the BCG vaccination Physical examination on admission showed that the patient was emaciated febrile and had moderate universal glandular enlargement hepatomegaly and splenomegaly and petechiae of the skin Chest X ray showed bilateral diffuse lung infiltration and there was anaemia granulocytopenia and thrombocytopenia A disseminated tuberculous infection was suspected and therapy was instituted with 20 mg/kg/24 h ethambutol 7 5 mg/kg/24 h rifampicin 7 5 mg/kg/24 h isoniazid and 2 mg/kg/24 h prednisone During treatment the patient s general condition improved she became afebrile gained weight the enlargement of liver and spleen decreased and the pulmonary infiltrations showed some regression However transient episodes with symptoms of increased intracranial pressure for one or two days recurred During the last of these incidents after 10 weeks of therapy the symptoms had progressed and therefore craniotomy was performed again The Pudenz shunt was found to be blocked by fibrin and was replaced but the cerebral damage was irreversible and the patient died

Immunological studies

The particle concentration of granulocytes in peripheral blood was 4 5×10⁹/l and 1 6×10⁹/l in January and August 1975 respectively The concentrations of immunoglobulins in serum in August 1975 were IgG 17 0 g/l IgA 1 7 g/l and IgM 1 1 g/l

The Moro tuberculin test was positive in January and also in August 1975 The intradermal tuberculin skin test with 1 TU tuberculin was positive (15 mm) in September 1975 No other skin tests for delayed hypersensitivity were performed

Table 1 *Results of immunological studies*

Date	Jan 75	Aug 75	Sept 75	Oct 75	Reference interval
Lymphocyte particle concentration in peripheral blood (10^9/l)	3 8	0 8	2 2	1 5	>1 5
% of lymphocytes					
T rosettes		65	65	75	52–80
B rosettes		31	16	13	8–29
Ig+cell		27	16	14	8–28
Results of lymphocyte transformation studie (increment counts per minute) stimulated with					
PHA		15 984	23 790	26 745	>17 000
PWM		7 134	7 056	7 191	> 5 000
PPD		452	2 651	2 972	> 1 000
Candida albicans		562	8 461	10 792	> 1 000
Staph aureus		156	1 430	2 949	> 1 000
E coli		88	1 154	2 967	> 1 000

The results of in vitro examination of lymphocytes are given in Table 1 T lymphocytes were measured by their spontaneous rosette formation with sheep erythrocytes (T rosettes) and B lymphocytes by rosette formation with human erythrocytes coated with antibody and complement C (B rosettes) as well as by direct immunofluorescence for surface immunoglobulins (Ig+ cells) Lymphocyte transformation tests were performed with the mitogens pokeweed mitogen (PWM) and phytohemagglutinin A (PHA) and the antigens tuberculin (PPD) *Candida albicans* (water soluble extract) *Staphylococcus aureus* and *Escherichia coli* (heat killed organisms) (19)

Concentration of complement factors C and C_4 in serum was normal

Autopsy findings

On macroscopical examination both lungs were dominated by diffuse granulated whitish firm infiltrates The liver weighed 1130 g (normally about 730 g) and the spleen 10 g (normally about 70 g) but both appeared to be macroscopically normal Lymph nodes approximately 1×1 cm in size and with normal consistency were found in the neck mediastinum and abdomen Thymus was normal in shape and weighed 3 6 g (normally about 10 g) The brain had a Pudenz shunt in place and was oedematous The dura was thickened and adherent in the posterior cranial fossa All other organs including bone marrow were normal

Microscopical examination of lungs liver kidney and pituitary showed typical epithelioid cell granulomas with Langhans giant cells and lymphocytes Ziehl Neelsen staining showed areas of necrosis with acid fast rods in the lungs The tubercles were identical with those seen in tuberculosis Except for those found in the pituitary there were no granulomas in the central nervous system and only fibrosis and infiltration of lymphocytes were seen in the leptomeninges and dura in the posterior fossa

The lymph nodes had a normal structure The thymus tissue was very sparse but of normal structure and Hassall s bodies were present

Bacteriological findings

Specimens of sputum gastric lavage urine blood bone marrow and spinal fluid examined for mycobacteria during the course of the disease before institution of treatment were negative by culture

Material taken at autopsy consisted of tissue from lung spleen liver kidney and brain as well as heart blood and faeces Culture on Lowenstein Jensen medium at 37 C gave growth of acid and alcohol fast rods resembling tubercle bacilli lungs 2 colonies spleen 16 colonies kidney 1 colony heart blood 1 colony There was no growth from the remaining specimens and particularly no growth from the brain tissue

Apart from a strong resistance to rifampicin the bacterial strain isolated could not be distinguished from BCG (5)

DISCUSSION

After intracutaneous vaccination BCG disseminates and multiplies until cellular immunity has developed (21) Epithelioid cell granulomas at the site of inoculation in the regional lymph nodes and in various distant organs can be found at least 2–3 years after vaccination without having caused any signs of disease and must be considered a normal phenomenon (10) Tubercle bacilli at this time however are found in sections of axillary lymph nodes only and cannot be isolated by culture (6)

Clinical disease caused by multiplication of BCG leading to generalized BCG itis has been

estimated to occur in 0 00001 % of those vaccinated (16) The total number of known cases of generalized BCG itis registered by the International Union against Tuberculosis is 41 31 of which were fatal (23) Not all of these have been published but from a review of 16 fatal cases available to us for study it would appear that all of these had occurred in patients with known or suspected immunodeficiency

Confirmed deficiency of cellular immunity either as part of a severe combined immunodeficiency syndrome (3 9 11 20) or as an isolated cellular immunodeficiency (14 17) was present in seven cases In all of these patients the histological findings in the affected organs differed from those seen as a response to BCG vaccination in immunocompetent individuals (10) Nodular tissue reaction formation of giant cells and occurrence of caseous necrosis were seen only infrequently or not at all and typical granulomas were not present Except for two cases of chronic granulomatous disease of childhood (7) the same histological picture was found also in the 7 other patients with fatal generalized BCG infection (1 2 4 8 12 13 18 22 24) Two patients in that group had hypogammaglobulinaemia (1 2 8) 4 had negative tuberculin tests (2 8 12 18) and one had lymphopenia (2) In none of these cases was the cellular immunity examined before death However since the histological findings were identical with those of patients with known cellular immunodeficiency and were different from those of normal individuals exposed to BCG it is probable that also these patients were immunodeficient

In the present case generalized BCG infection was confirmed by isolation of BCG from several organs on autopsy and there was no evidence of immunodeficiency A history of uncomplicated measles and a normal response to smallpox vaccination is firm evidence against any significant cellular immunodeficiency and there was no previous history of frequent or severe infections The local response to BCG vaccination was normal and the patient had a positive tuberculin skin test

In vitro tests for humoral and cellular immunity were normal The lymphopenia and the slight response to transformation with mitogens and antigens in August 1975 may well be explained by the overwhelming infection then existing (25) The thymus was small but microscopically the tissue though sparse was normal and Hassall s bodies were present The hypoplasia found is as could be expected after more than 2 months treatment with corticosteroids The tissue response to the tuberculou infection was normal with the occurrence o typical welldemarcated epithelioid cell granulomas A complete study of humoral immunit by estimation of the response to specific antigens and a study of all complement factors an granulocyte function were not carried out bu the information available provides no suspicio of any defect in these systems We believe therefore that this is an example of generalized BCG infection in a patient who by th criteria normally employed would be considered to be fully immunocompetent

The occurrence of an epithelioid cell granu loma 22 months after BCG vaccination at place other than the inoculation site is not ur usual (10) However reports of granuloma occurring in and primarily causing symptom from the central nervous system are nc known to us In a case published by Veith e al (19) symptoms from the CNS after BC vaccination appeared to have been secondar to thrombosis of cerebral veins and sinuses and in a case reported by Watanabe et al (24 cerebral tuberculomas were found only afte 10 years generalized BCG infection

The reason for the development of the generalized BCG infection is not known but it ma be assumed that the Pudenz shunt facilitate bacterial dissemination

The clinical effect of the antituberculou therapy and the small number of bacilli foun by culture of the autopsy material point to a least partial control of the infection at time o death The cause of death must therefore be attributed to the unfortunate manifestation o the disease in the CNS

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CASE REPORT

KAWASAKI DISEASE IN KUWAIT

A Report of Two Cases

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ABSTRACT Majeed H A and Olson I A (Department of Paediatrics The Chest Hospital and Department of Human Morphology and Experimental Pathology Kuwait University Medical School Kuwait) *Kawasaki disease in Kuwait A report on two cases Acta Paediatr Scand 67 525 1978*—Kawasaki disease was *first reported in Japan in 1967* Since then it has been reported from the United States South Korea Greece Canada Australia Scandinavia and Scotland Two cases of Kawasaki disease are presented from Kuwait and believed to be the first report of the disease from the Arab world

KEY WORDS Kawasaki disease infantile polyarteritis nodosa mucocutaneous lymph node syndrome

In 1967 Kawasaki described a febrile mucocutaneous disease affecting infants and children which he designated as mucocutaneous lymph node syndrome (MLNS) (8) The Japanese MLNS study group has recently adopted the name Kawasaki disease thus accepting it as a nosological entity (9) Outside Japan the disease has so far been reported from the United States South Korea Greece Canada (17) Australia (11) Scandinavia (1) and Scotland (20)

This report describes two cases of Kawasaki disease in Kuwait and is believed to be the first report of the disease from the Arab world

CASE HISTORIES

Case 1 A S a ten-year-old Kuwaiti boy presented on May 1975 with fever headache abdominal pain and arthralgia of both knees of three days duration which had not responded to oral penicillin Examination showed an ill looking child who was unable to walk because of pain in both knees which were not red or swollen Temperature was 39 °C The throat and oral cavity were congested with a post natal discharge no vesicles were noted The conjunctivae were mildly congested lips dry and the neck was stiff There was bilateral anterior cervical lymph adenitis A discrete maculo-erythematous rash involved the trunk and extremities the palms and soles showed diffuse erythema Laboratory blood examination showed haemoglobin 8 [redacted] mmol/l leucocytosis (12×10^9/l) with neutrophilia platelets normal and erythrocyte sedimentation rate of *30 mm/h and positive C reactive protein* Throat culture grew no organisms ASO titre was 100 Todd units and rheumatoid factor negative C S F was normal and urine microscopy revealed no deposits Blood urine and C S F cultures were sterile Widal and Paul Bunnel tests were negative Chest X ray E C G blood urea serum electrolytes and proteins were all normal SGPT and alkaline phosphatase were normal but SGOT was mildly elevated Oral penicillin was continued On the 4th day reddening of the palms and soles were more obvious with no induration and lips fissured On the 7th *day temperature started settling down* conjunctivae were normal the rash was fading although the palms and soles were still mildly erythematous and the child could walk with no joint pains On the 9th day the temperature was normal and on the 17th day he was completely well apart from the characteristic desquamation of palms and soles near the finger tips and along the sides of the nails Follow up for two years showed no recurrences and normal cardiovascular system

Case 2 [redacted] H a 5 year-old English boy who had arrived in Kuwait from England six weeks before presented on *April 1977 with fever and unilateral painful* anterior cervical adenitis of two days duration Examination showed an ill looking child temperature was 40 3 C The throat and oral cavity were severely congested with post



Fig 1 Erythema of the palms with the characteristic desquamation in Case 2

nasal discharge no vesicles were seen The left tonsillar lymph gland was tender and enlarged (5×2 cm) He was treated with oral penicillin but within twelve hours (two doses) developed discrete maculo erythematous rash over the trunk and extremities Penicillin was stopped and oral clindamycin started By the fifth day while still pyrexial he developed a severe conjunctival congestion diffuse erythema over the nose and anterior malar facial area marked impressive diffuse erythema and induration of palms and soles and reddening and fissuring of the lips Laboratory blood examination showed haemoglobin 6 5 mmol/l leucocytosis (18 7×10⁹/l) with marked neutrophilia platelets normal erythrocyte sedimentation rate 110 mm/h and positive C reactive protein LE cell and Coomb s tests were negative Total serum protein 76 g/l (albumin 35 g/l and globulins 41 g/l) SGOT and alkaline phosphatase were normal but LDH was mildly elevated Microscopical examination of urine showed leucocytes 12/H P F and urine culture was sterile Tuberculin test was negative Throat culture grew no organisms and ASO titre was 50 Todd units Chest X ray and E C G were normal A diagnosis of Kawasaki disease was made On the 7th day the rash started fading but the child continued to be pyrexial developed neck stiffness pain and swelling of both knees and ankles and he looked more ill Prednisone orally 40 mg/day was started In 48 hours (9th day of illness) there was a dramatic improvement temperature settled down to normal neck stiffness and arthritis disappeared and the child looked well Prednisone was stopped gradually over two weeks On the 13th day he started the characteristics desquamation from palms and soles near the finger tips and along sides of nails (Fig 1) On the 25th day while still desquamating from finger tips and along sides of nails transverse ridges appeared on the nails Follow up for six months showed no recurrences and normal cardiovascular system

DISCUSSION

Kawasaki disease has been differentiated from scarlet fever and Steven Johnson syndrome (10) and its clinical pattern clearly outlined The combination of marked impressive erythema of palms and soles intense conjunctivitis non suppurative cervical adenitis in the acute stage with the characteristic membranous desquamation of fingers and toes in the convalescent stage in the course of a febrile exanthematous childhood disease should make a confident diagnosis of Kawasaki disease possible Our patients fitted well with this characteristic clinical pattern which seems to be different from all other childhood exanthemata (5) They are however in the upper age group for the disease (10) They both developed transient neck stiffness and joint involvement originally described by Kawasaki et al as significant findings which are important for the diagnosis in combination with the principal symptoms (10) examination of the C S F in one of our patients revealed normal findings The second child was English who arrived from England six weeks before he became ill in Kuwait The disease has recently been reported from Britain (20)

Of current interest in the relationship between Kawasaki disease (KD) and infantile polyarteritis nodosa (IPN) Fetterman & Roberts in 1963 (18) reviewed 20 cases of IPN and outlined a clinical syndrome which showed clinical resemblance to KD (4 10) The findings of widespread arteritis involving the brachial iliac mesenteric renal testicular and other arteries in cases of KD (21) suggests that this may be a vasculitis syndrome (7) Polyarteritis nodosa has been classified as a subtype of necrotising angiitis (12) The infantile form is characterised by the high incidence of coronary artery involvement (18) Recent studies on KD have shown similar findings Tanaka et al (21) carried out autopsy studies on 29 cases of fatal MLNS the incidence of coronary artery involvement was 100% and the vascular pathological findings

ere identical with IPN Kato et al (7) studied e non fatal clinical form of MLNS they per ormed coronary angiography on 20 patients ho survived the illness and found abnormal ngiograms in 12 (60%) Recently the Japa ese research committee on KD (11) found hat of 321 cases given coronary angiography 3% showed abnormal angiograms Asai et al 2) reported E C G abnormalities and/or car liac enlargement in 70% in the acute stage Physical examination of our patients showed no abnormal clinical cardiac findings and their plain chest X rays and E C G tracings were normal

The clinical and pathological close simi larities between KD and IPN have not escaped the attention of Kawasaki et al (10) Fetter man & Roberts (4) and Tanaka et al (21) and have rightly tempted Ahlstrom et al (1) and Smith (20) to suggest that the two diseases may in fact represent one entity Similar though possibly less striking clinico patho- logical overlaps are known to occur amongst certain subtypes of necrotising angiitis (poly arteritis nodosa allergic angiitis and collagen disease angiitis) (3) A familiar example is the problem of differentiating the most severe forms of Henoch Schonlein s purpura (H S) from polyarteritis nodosa (PA) where there is considerable clinical overlap and pathological distinction may not be possible (16) and sug gestions were in fact made to consider H S and PA as presentations of the same disease (16) Yet we are impressed by the close clinico-pathological similarities between IPN and the fatal form of KD (21) and the high in cidence of coronary arteritis in the non fatal clinical form of KD (7 11) It may be that the fatal form of KD and IPN are probably one entity representing one end of a disease pro- cess that tends to occur in young babies while the more common non fatal clinical form of KD which tends to occur in older children represents the other end

The aetiology of KD is still obscure The Group A Beta haemolytic streptococcus was ruled out as the causative agent (10) this fitted with our observations in Kuwait where though rheumatic fever has been fairly com mon (15) our two patients are the first and only report of KD from the country The find ing of rickettsia like bodies in patients with KD (6) was not supported by other reports (19) and the unresponsiveness of the disease to antibiotics failed to confirm the rickettsial aetiology

Hypersensitivity mechanism is implicated in the adult form of PA (20) and recently elevated serum levels of IgE have been reported in both KD and IPN (13 14) The results of a com parative study of adult polyarteritis nodosa and IPN that does not show the MLNS mani festations (21) will be awaited with interest

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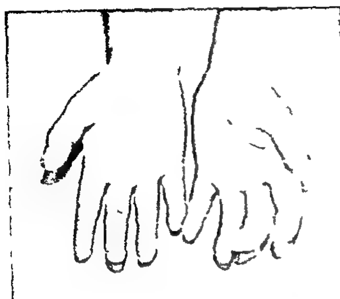


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CASE REPORT

MALFORMATION OF THE GREAT VEIN OF GALEN WITH NEONATAL HEART FAILURE

Report of Two Cases

J EIDE and M FØLLING

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ABSTRACT Eide J and Følling M (The Gade Institute Department of Pathology and the Department of Clinical Physiology Haukeland Hospital University of Bergen Bergen Norway) Malformation of the great vein of Galen with neonatal heart failure Report of two cases Acta Paediatr Scand 67 529 1978 —The clinical and pathological findings in two neonates with the malformation of the great vein of Galen are given They both reported with serious neonatal heart failure suggesting congenital heart disease In one of them cardiac catheterization revealed a foetal pattern of circulation causing cyanosis A bruit and in one of them a thrill over the skull gave the clinical diagnosis of an intracranial arteriovenous aneurysm They died 48 and 144 hours after birth in spite of medical treatment The outlook for patients having malformation of the great vein of Galen and suffering neonatal heart failure treated conservatively seems hopeless

KEY WORDS Arteriovenous aneurysms malformation of the great vein of Galen neonatal heart failure

The vein of Galen malformation is said to be present when one or more major branches of arteries of the carotid or vertebral system feed directly into a hugely dilated vein of Galen (5 13) which functions as an arteriovenous aneurysm short circuiting varying and sometimes large volumes of blood and giving rise to heart failure and/or various neurological symptoms (5 7 8 16) In the neonatal period it may cause fatal heart failure The diagnosis is usually made post mortem as the symptoms closely mimic a serious congenital heart defect (1 5) During the last 7 years we have seen two cases and report the clinical and post mortem findings as they may contribute to the clinical diagnosis of future cases and thus open up the possibility of adequate treatment

CASE REPORTS

Patient 1

A H a newborn female was born after 40 weeks gestation The mother had had one previous first trimester abortion She had been given diazepam in the third month of pregnancy because of vaginal haemorrhage and an antibiotic in the 7th month for urinary infection The labour was normal and lasted for 7 hours Birth weight was 3 150 g and crown–heel length 48 cm The head circumference measured 34 cm

The child was cyanotic with tachycardia tachypnoea and hepatomegaly from birth A grade ?–3/6 systolic murmur that later disappeared was heard in the third intercostal space on the left side of the sternum Electrocardiogram and chest X ray indicated cardiomegaly with a right ventricular and right atrial predominance Cardiac catheterization was performed The left side of the heart was not entered by the catheter Right atrial pressure was high and the right ventricular and pulmonary arterial pressure were at systemic levels Right ventricular angiography showed an enlarged right ventricle widely open ductus arteriosus and right to left shunting to the descending aorta The pulmonary circulation was very slow and the left side of the heart could not be visualized until 5 seconds after the contrast injection

The findings were misinterpreted as hypoplastic left heart syndrome The day after catheterization a loud bruit was heard over the skull and an intra cranial arteriovenous malformation was suggested as the cause of the cardiac failure The child died 48 hours after birth not responding to medical treatment

Autopsy (0 553/59) revealed a malformation of the vein of Galen (Fig 1) bilaterally fed by branches of the pos-

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Fig 3 T Pathological specimen Base of brain and cerebellum posterior view The aneurysm of the vein of Galen is filled with water from a catheter to demonstrate its size

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The frequency of this malformation is not precisely known It seems comparatively low but is not insignificant 15 4% of the congenital intracranial arteriovenous malformations in Lagos & Riley s (10) series 50% in Gold et al (5) the arteriovenous aneurysms comprising about 1 4% of all verified intracranial tumours (4) and 13 9% of all intracranial aneurysms (14) Gold et al (5) were able to find 35 cases in the literature and reported another 5 Amacher & Shillito (1) in their review from 1973 accepted 37 cases after critical analysis of reports in the English language considering only the solitary aneurysms and not the secondary dilatations in which an angiomatous malformation drains through the Galenic system (11) Eleven cases belonged to the neonatal heart failure group

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The question of surgical treatment of pa



Fig 1 A H Pathological specimen Base of brain the cerebellum and brain stem removed Tortuous vessels can be seen on the right side connecting the posterior cerebral artery to the dilated vein of Galen (*arrow*)



Fig 2 T B Chest X ray anterior view showing marked cardiac enlargement

terior cerebral arteries 3 on the right side and 1 on the left The vein showed a large sac like dilatation with compression of surrounding brain structures The heart showed no congenital defects but marked right ventricular and atrial hypertrophy and dilatation The calibre of the aortic arch the brachiocephalic trunk the common internal and external carotid arteries were enlarged and the ductus arteriosus was open The lungs showed incipient bronchopneumonia and the lungs liver spleen and kidneys evidence of passive congestion

Patient 2

T B a newborn male was born after 40 weeks gestation Labour exceeded 24 hours but was uncomplicated The infant weighed 3 600 g and had a crown–heel length of 55 cm Head circumference was 35 cm Apgar score was 9 after 1 and 5 min

Progressive respiratory distress developed 48 hours after birth Chest X ray showed cardiomegaly and the infant was transferred to this hospital

At admission he was in severe heart failure with enlarged liver 4 cm below the costal margin No cyanosis Heart rate was 150/min with a normal femoral pulse and blood pressure 65/40 in upper and lower limbs Respiratory frequency increased from 70/min to 95/min during the ensuing 6 hours A grade 2–3/6 systolic murmur was heard along the left costal margin The electrocardiogram showed QRS complexes within normal limits and p-waves suggesting right atrial enlargement Chest X ray revealed an enlarged heart with predominance of the right side (Fig 2)

A loud coarse intracranial continuous bruit and a thrill over the skull led to the clinical diagnosis of a large intracranial arteriovenous aneurysm The neurosurgeon was consulted but the lesion was considered inoperable because of the child s clinical condition Heart catheterization and cerebral angiography were therefore not performed The progressive heart failure did not respond to treatment and he died 144 hours after birth

Autopsy (0 250/76) revealed a malformation of the vein of Galen with a huge sac like dilatation that made large impressions on the medial side of the hemispheres and compressed the roof of the midbrain the posterior part of the corpus callosum and the superior part of the cerebellum The ventricular system of the brain did not seem to be dilated Three to four branches of the posterior cerebral arteries on each side fed into the aneurysm (Fig 3) The torcula Herophili the straight and sigmoid sinuses were dilated as were the grooves for the sinuses on the internal surface of the skull The heart showed enlargement predominantly on the right side Dilatation of the pulmonary artery the aorta and the neck arteries were noted The ductus arteriosus was patent and no congenital heart defects were found

DISCUSSION

A widely dilated sac like vein of Galen fed directly from few large branches of the pos



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tients belonging to the neonatal heart failure group has emerged only recently, as the condition has only rarely been diagnosed before death One such case reported by Gomez et al (6) was treated with ligature of the right common carotid artery later with ligature of the left external carotid artery and recovered from the heart failure However the cerebral condition and subsequent course were not mentioned One patient was reported by Amacher & Shillito (1) and was diagnosed clinically by an intracranial bruit cerebral angiography and cardiac catheterization Craniotomy and partial ligation of the feeder arteries was carried out on the fourth day of life in spite of serious heart failure The infant died 18 hours later of heart failure that did not respond to intensive measures The major postoperative hazard in this clinical group seems to be progressive heart failure following closure of the shunt Immediate phlebotomy digitalization and rigid fluid control may be mandatory (5)

The effectiveness of surgery in the neonatal heart failure group remains to be settled as the evidence so far is very limited However it seems reasonable to try surgical measures as the evidence points to an invariable fatal outcome in conservatively treated cases One also has to consider rewarding results of surgery with other forms of intracranial arteriovenous aneurysms and with the same malformation in other age groups as shown by Amacher & Shillito (1) Gold et al (5) and Cronqvist et al (3)

Diagnosis depends on examination for and awareness of the significance of an intracranial bruit when examining neonates with heart failure and suspected congenital heart disease The bruit is loud and pulse synchronous and differs from the soft bruits that may be heard in normal children (12)

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CASE REPORT

THE ABSENCE OF FACTOR II IN A CHILD WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT Lillquist K B Dyerberg J and Krogh Jensen M (Dept of Paediatrics Dept of Clinical Chemistry Section of Blood Coagulation and Dept of Medicine Section of Haematology Aalborg Hospital Denmark) The absence of factor II in a child with systemic lupus erythematosus Acta Paediatr Scand 67 533 1978.—This report describes a patient with active systemic lupus erythematosus (SLE) who developed haemorrhagic diathesis due to a lowering of plasma factor II activity No evidence was found suggesting a plasma inhibitor of factor II The present case indicates that in some patients with SLE factor II activity may be low or completely absent due to impairment of factor II synthesis further that prednisone but not azathioprine may ameliorate this defect

KEY WORDS Factor II deficiency haemorrhagic diathesis systemic lupus erythematosus children

Patients with systemic lupus erythematosus (SLE) often develop haemorrhagic diathesis due either to thrombocytopenia or circulating anticoagulants against specific coagulation factors (3) However a patient with SLE has bee reported recently who had severe bleeding due to an acquired deficiency of factor II but in whom there was no evidence of a circulating inhibitor (10) We report a new case of factor II deficiency in a patient with SLE in whom no circulating anticoagulant could be demonstrated

METHODS

Blood samples for the coagulation studies were collected in plastic tubes from a cubital vein with minimal stasis after discarding the first 1–2 ml of blood Tri sodium citrate 3 8% w/v one part to nine parts of venous blood was used as an anticoagulant Immediately after withdrawal of the blood the tubes were placed in iced water until the plasma could be separated by centrifugation this was carried out less than half an hour after sampling

Coagulation assays were performed within either one hour of centrifugation in which case the plasma was kept at 0°C or after storage of −60°C Coagulation analyses including assays for single coagulation factors were performed as described by Biggs (1) and by Nilsson (12 13) Factor II assay was performed by recalcifying a mixture of diluted patient plasma and human factor II deficient plasma (Merz and Dade Bern Switzerland) Reference plasma Ci Trol (Merz and Dade) was used for the calibration curve Factor II related antigen was determined by rocket immunoelectrophoretic assay as described by Laurell (7) using antibody against factor II (Clot Immun® Prothrombin) from Behring Werke (Marburg West Germany) Assays for circulating anticoagulants were performed either semiquantitatively as a screening test or quantitatively during the determination of anticoagulant activity against factor II (13) The principle in the semiquantitative assay is that the recalcification time is measured after patient plasma in varying quantities has been incubated with normal plasma

The test for determining anticoagulant activity against factor II consisted of assaying factor II activity in a test system consisting of one part of normal plasma one part of patient plasma and one part of factor II deficient plasma The results of this were compared with the activity of factor II in a test system where the patient plasma had been replaced by buffer Incubation was performed at 37°C for 2 min as well as for 30 min

CASE HISTORY

The patient was a 12 year-old boy with no family history of haemorrhagic anomalies The pregnancy and delivery had been normal Attacks of febrile convulsions devel

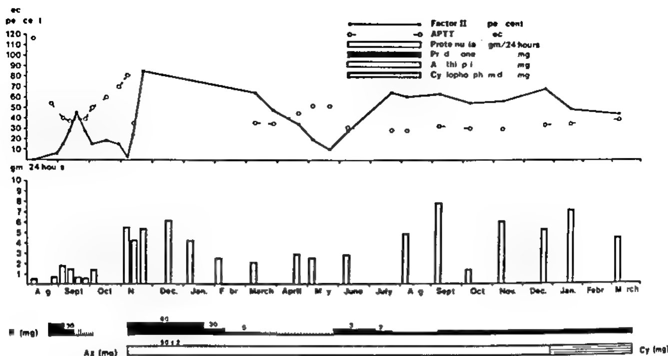


Fig. 1. Changes in factor II and APTT and the trend of proteinuria during prednisone azathioprine and cyclophosphamide treatment

oped during the first years of life and were treated with hydantoin from 1965 to May 1975 At that time the patient was admitted to hospital suffering from a rise in temperature headache muscular and abdominal pains Numerous cafe au lait spots were found all over the skin as well as many partially healed ecchymoses on the lower extremities Coagulation tests revealed prolonged activated partial thromboplastin time (APTT) of 64 sec (norm 25–40 sec) P-P test of 55% (norm 85–100%) factor VIII 130% (norm 60–160%) and factor IX 45% (norm 60–140%) Bleeding time (Ivy) 11 min (norm 6–12 min) platelets 420×10 /l (norm 140–340×10 /l)

The case was regarded as a mild hemophilia B even though the coagulation tests provided no actual explanation of the bleeding tendency A supplement of vitamin K given i m had no effect on the prothrombin time The symptoms disappeared spontaneously and the patient was discharged in June 1975

In August 1975 the patient was readmitted to the hospital because of pain tenderness and swelling of the elbow and knee joints as well as of the parotid glands ecchymoses and suspected melena

The physical examination revealed a pale and debilitated patient His blood pressure was 120/90 mmHg There were multiple ecchymoses on his extremities and trunk a hematoma on the inner side of the left arm and hemarthrosis of both elbow joints Splenomegaly was present but no hepatomegaly A chest X ray showed enlarged mediastinal nodes but no enlargement of the peripheral lymph nodes A reddish erythema appeared on the trunk and upper extremities a few days after admission

The results of the following laboratory tests were normal leukocyte count reticulocyte count platelets serum glutamic pyruvic transaminase (SGPT) serum lactic dehydrogenase (LDH) alkaline phosphatase serum bilirubin serum albumin ceruloplasmin haptoglobin antistreptolysin O and antistreptococcus hyaluronidase The Wassermann reaction (WR) Rose Waaler test and LE-cell test were all negative

Hemoglobin was found to be 4 9 mmol/l and the sedimentation rate 135 mm/h Serum protein electrophoresis showed increased gammaglobulin Both the test for antinuclear factor and Coombs test were strongly positive The DNA antibody titre was increased and immunofluorescent studies of a skin biopsy revealed granular immune deposits consisting of IgG and IgM in the dermo-epidermal layers and IgG deposits in the vessel walls Microscopic hematuria and proteinuria were present indicating renal disease The clinical picture and the laboratory findings were compatible with SLE

The results of the coagulation tests showed prolongation of APTT to 116 sec a P-P test of 39% an almost total absence of factor II (<1%) (norm 60–120%) a factor II related antigen concentration of 5% of normal factor V 51% (norm 80–120%) factor VII 100% (norm 80–120%) factor VIII 205% factor IX 25% factor X 60% (norm 80–120%) bleeding time (Ivy) 15 min and platelets 214×10^9/l Fibrinogen 15 µmol/l (norm 5–12 µmol/l) fibrinogen degradation products (FDP) 40–160 mg/l (norm 0–10 mg/l) fibrin monomers 0 (norm 0)

Coagulation studies in our laboratory as well as in the coagulation laboratory in Malmo failed to reveal any circulating antibodies against factor II The patient was treated with prednisone this resulted in a normalization of the coagulation abnormalities though the abnormalities reappeared following withdrawal of the drug (Fig 1) An

attempt was made to supplement the steroid treatment with azathioprine (100 mg daily) and at the same time the steroid dosage was reduced. However this resulted in a fall in factor II and an aggravation of the renal disease (Fig. 1). From August 1976 the child was given 15–20 mg of prednisone daily; this treatment maintained factor II levels between 50% and 60%. As the proteinuria increased, the administration of azathioprine was discontinued and cyclophosphamide given in its stead. The patient was again admitted to hospital in March 1977 with malignant hypertension and died within a few days of congestive heart failure. Post mortem examination revealed chronic proliferative glomerulonephritis—LED-nephritis.

DISCUSSION

The activity of factor II was below 1% of normal before treatment was commenced. Factors V, IX and X were slightly reduced. Treatment with prednisone resulted in a rapid normalization of all three coagulation factors. Prednisone was withdrawn twice and on both occasions this was followed by a rapid decrease in factor II activity together with a slight reduction in factors V and IX. Urinary loss of coagulation factors has been reported in association with the nephrotic syndrome but severe proteinuria (>7 g daily) has been present in nearly all of these cases (4, 11). In the majority of patients this was in fact a renal loss of factor IX, and the slight reduction in factor IX in our patient may have been caused by such renal loss.

Azathioprine did not seem to have any effect on the clinical course or the laboratory findings. The cause of the factor II deficiency is unknown. Therapy with vitamin K had no effect on factor II concentration.

Increased consumption of factor II is seen in disseminated intravascular coagulation. However, neither clinical nor obvious laboratory evidence of this condition was present. A low level or complete absence of factor II due to the presence of circulating anticoagulants has been demonstrated in several patients with SLE (5, 6, 8, 9, 11) and Corrigan et al. reported a patient with the presence of an anticoagulant associated with an isolated deficiency of factor II (2).

However, in the present patient in vitro studies demonstrated the absence of immunoreactive factor II and no plasma inhibition of factor II activity. Furthermore, when factor II (Preconativ® Kabi) was given to the patient in a dosage of 500 units, both factor II and APTT were normalized for 24 hours. This finding also appears to exclude the possibility of a circulating anticoagulant.

In 1976 Natelson et al. reported a patient with absent factor II in whom a circulating anticoagulant could not be demonstrated (10). A normal factor IX activity was present in that patient. The study of Natelson et al. and the present findings suggest that factor II activity may be low or absent entirely owing to an impaired synthesis in some patients with SLE and that prednisone—but not azathioprine—may ameliorate this defect.

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The analysis in the coagulation laboratory in Malmö was kindly performed by Prof. I. M. Nilsson. The technical assistance of E. Stoffersen is highly appreciated.

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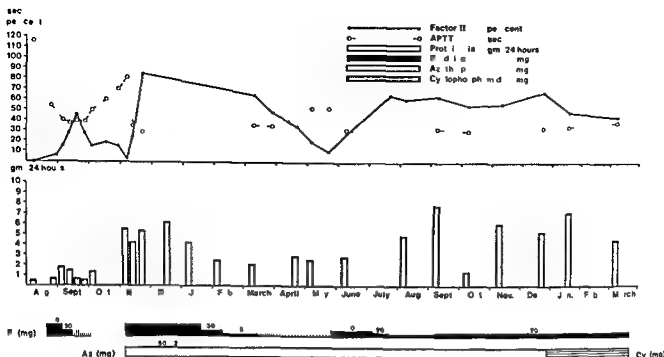


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The results of the coagulation tests showed prolongation of APTT to 116 sec a P–P test of 39% an almost total absence of factor II (<1%) (norm 60–120%) a factor II related antigen concentration of 5% of normal factor V 51% (norm 80–120%) factor VII 100% (norm 80–120%) factor VIII 205% factor IX 25% factor X 60% (norm 80–120%) bleeding time (Ivy) 15 min and platelets 214×10⁹/l Fibrinogen 15 μmol/l (norm 5–12 μmol/l) fibrinogen degradation products (FDP) 40–160 mg/l (norm 0–10 mg/l) fibrin monomers 0 (norm 0)

Coagulation studies in our laboratory as well as in the coagulation laboratory in Malmo failed to reveal any circulating antibodies against factor II The patient was treated with prednisone this resulted in a normalization of the coagulation abnormalities though the abnormalities reappeared following withdrawal of the drug (Fig 1) An

attempt was made to supplement the steroid treatment with azathioprine (100 mg daily) and at the same time the steroid dosage was reduced. However, this resulted in a fall in factor II and an aggravation of the renal disease (Fig. 1). From August 1976 the child was given 15–20 mg of prednisone daily; this treatment maintained factor II levels between 50% and 60%. As the proteinuria increased, the administration of azathioprine was discontinued and cyclophosphamide given in its stead. The patient was again admitted to hospital in March 1977 with malignant hypertension and died within a few days of congestive heart failure. Post mortem examination revealed chronic proliferative glomerulonephritis–LED-nephritis.

DISCUSSION

The activity of factor II was below 1% of normal before treatment was commenced. Factors V, IX and X were slightly reduced. Treatment with prednisone resulted in a rapid normalization of all three coagulation factors. Prednisone was withdrawn twice and on both occasions this was followed by a rapid decrease in factor II activity together with a slight reduction in factors V and IX. Urinary loss of coagulation factors has been reported in association with the nephrotic syndrome but severe proteinuria (>7 g daily) has been present in nearly all of these cases (4, 11). In the majority of patients this was in fact a renal loss of factor IX, and the slight reduction in factor IX in our patient may have been caused by such renal loss.

Azathioprine did not seem to have any effect on the clinical course or the laboratory findings. The cause of the factor II deficiency is unknown. Therapy with vitamin K had no effect on factor II concentration.

Increased consumption of factor II is seen in disseminated intravascular coagulation. However, neither clinical nor obvious laboratory evidence of this condition was present. A low level or complete absence of factor II due to the presence of circulating anticoagulants has been demonstrated in several patients with SLE (5, 6, 8, 9, 11) and Corrigan et al. reported a patient with the presence of an anticoagulant associated with an isolated deficiency of factor II (2).

However, in the present patient, in vitro studies demonstrated the absence of immunoreactive factor II and no plasma inhibition of factor II activity. Furthermore, when factor II (Preconativ® Kabi) was given to the patient in a dosage of 500 units, both factor II and APTT were normalized for 24 hours. This finding also appears to exclude the possibility of a circulating anticoagulant.

In 1976, Natelson et al. reported a patient with absent factor II, in whom a circulating anticoagulant could not be demonstrated (10). A normal factor IX activity was present in that patient. The study of Natelson et al. and the present findings suggest that factor II activity may be low or absent entirely owing to an impaired synthesis in some patients with SLE and that prednisone—but not azathioprine—may ameliorate this defect.

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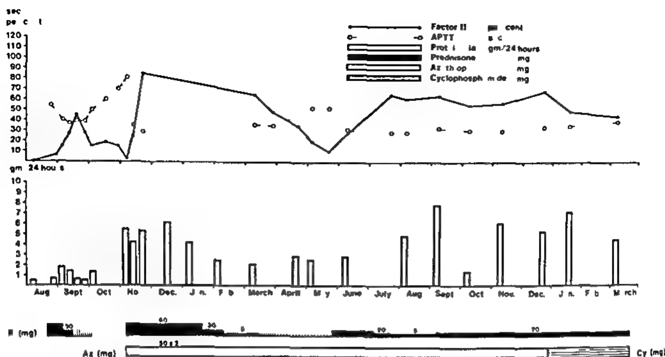


Fig 1 Changes in factor II and APTT and the trend of proteinuria during prednisone azathioprine and cyclophosphamide treatment

oped during the first years of life and were treated with hydantoin from 1965 to May 1975 At that time the patient was admitted to hospital suffering from a rise in temperature headache muscular and abdominal pains Numerous cafe au lait spots were found all over the skin as well as many partially healed ecchymoses on the lower extremities Coagulation tests revealed prolonged activated partial thromboplastin time (APTT) of 64 sec (norm 25–40 sec) P–P test of 55% (norm 85–100%) factor VIII 130% (norm 60–160%) and factor IX 45% (norm 60–140%) Bleeding time (Ivy) 11 min (norm 6–12 min) platelets 420×10⁹/l (norm 140–340×10⁹/l)

The case was regarded as a mild hemophilia B even though the coagulation tests provided no actual explanation of the bleeding tendency A supplement of vitamin K given i m had no effect on the prothrombin time The symptoms disappeared spontaneously and the patient was discharged in June 1975

In August 1975 the patient was readmitted to the hospital because of pain tenderness and swelling of the elbow and knee joints as well as of the parotid glands ecchymoses and suspected melena

The physical examination revealed a pale and debilitated patient His blood pressure was 120/90 mmHg There were multiple ecchymoses on his extremities and trunk a hematoma on the inner side of the left arm and hemarthrosis of both elbow joints Splenomegaly was present but no hepatomegaly A chest X ray showed enlarged mediastinal nodes but no enlargement of the peripheral lymph nodes A reddish erythema appeared on the trunk and upper extremities a few days after admission

The results of the following laboratory tests were normal leukocyte count reticulocyte count platelets serum glutamic pyruvic transaminase (SGPT) serum lactic dehydrogenase (LDH) alkaline phosphatase serum bilirubin serum albumin ceruloplasmin haptoglobin antistreptolysin O and antistreptococcus hyaluronidase The Wassermann reaction (WR) Rose Waaler test and LE cell test were all negative

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CASE REPORT

NATURAL HISTORY OF JUVENILE RHEUMATOID ARTHRITIS

A Follow up Study of a Case with Special Reference to Clinical Electroencephalographic and Neuropathological Findings

M SILLANPAA A [redacted] LANG and H KALIMO

From the Departments of Paediatrics Clinical Neurophysiology and Pathological Anatomy University of Turku Turku Finland

ABSTRACT Sillanpaa M Lang A H and Kalimo H (Departments of Paediatrics Clinical Neurophysiology and Pathological Anatomy University of Turku Turku Finland) Natural history of juvenile rheumatoid arthritis A follow up study of a case with special reference to clinical electroencephalographic and neuropathological findings Acta Paediatr Scand 67 537 1978.—A detailed comparison between the clinical and EEG findings is made in a case of a boy with juvenile rheumatoid arthritis (JRA) who died at [redacted] years 6 5 years after the beginning of the follow up period In the course of the disease seven EEG recordings were made showing a progressive diffuse slowing and disorganization with some improvement during short remissions In relapses diffuse slowing was associated with grave asymmetries in the EEG which however fluctuated and later disappeared without accompanying clinical or neuroradiological abnormalities An abundancy of different residual findings however remained in the EEG after relapses There were spike-and wave paroxysms in every record except at the terminal stage A stepwise slowing and disorganization was also seen in these paroxysms as background activity The final cause of death was an intraventricular haemorrhage No cerebral amyloidosis was found at autopsy In conclusion it [redacted] suggested that JRA is also a brain disease manifested as a cerebral vasculitis

KEY WORDS Juvenile rheumatoid arthritis cerebral symptoms follow up study EEG

In a retrospective study of one hundred children with juvenile rheumatoid arthritis (JRA) (4) a rich variety of EEG abnormalities were found in about half the cases The EEG changes could not be attributed to reactions of the C N S to fever or medical treatment A hypothesis was put forth that the abnormalities are derived from [redacted] primary cerebral process associated with JRA probably a vasculitis of the cerebral vessels

In order to obtain more information on the cerebral pathophysiology of JRA we have made a detailed follow up study of a case suffering from this disease The follow up period lasted from the onset of the disease up until the death of the patient six and a half years

CASE HISTORY

Boy born 23 12 1960 was admitted [redacted] the Department of Paediatrics University Hospital of Turku Sept 1969 because of fever sore throat pain in the right shoulder and migratory exanthema in different parts of the body There were migratory joint symptoms high erytrocyte sedimentation rate and marked malaise but the symptoms initially subsided with antirheumatoid treatment Later however he had to be admitted to hospital altogether [redacted] times due to the fluctuating course of the disease with mainly short remissions and often long lasting relapses The general condition although temporarily improved gradually deteriorated At the final stage an increasing weakness of right sided extremities and right corner of the mouth disturbed speech and swallowing were found Massive generalized oedema showed no response to treatment and the patient decreased 16 03 76

EEG recording and analysis

EEG recording was carried out seven times during the disease The international 10–20 system of placing electrodes was adapted and both bipolar and common ref

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Fig 4 The intracerebral blood vessels were thin walled without evidence of amyloid deposition not even in the area of the subacute intraparenchymatous haemorrhage in the right basal ganglia Van Gieson stain Bar 300 μm



Fig 5 Mainly mononuclear inflammatory infiltrate ad herent to the wall of a vein in the midbrain with imminent perivascular spread Van Gieson stain Bar 100 μm

erence derivations were used Flash light stimulation was routinely used The frequency of the dominating parieto-occipital rhythm was measured from numerous repre sentative samples of records during the state of high vigilance level e g immediately after eye closing The essential EEG findings of the consecutive records are presented in Table 1 A representative sample of two first and the last recordings are seen in Figs 1 A and B 2 and 3 respectively

Autopsy findings

The deceased was cachectic with severe joint deformities He had severe generalized amyloidosis which affected practically all internal organs The brain was slightly swollen In the basal ganglia there was subependymatous haemorrhage with subsequent rupture into the ventricular system Due to an accident the brain was discarded with only two samples saved for microscopy one from the area of the haemorrhage the other from the midbrain The meningeal and intra cerebral blood vessels were thin walled with no Congo red positive amyloid material (Fig 4) There was no definite evidence of acute vasculitis in the samples available However some abnormal intra vascular collections of chiefly mononuclear inflammatory cells were seen adherent to the walls of a couple of veins in the midbrain (Fig 5) A few small perivascular infiltrates of mononuclear cells were also noted The sample from the basal ganglia displayed the intraparenchymatous haemorrhage (Fig 4)

DISCUSSION

In a reprospective analysis of 100 children with JRA (4) different EEG abnormalities were common such as abundant beta activity paroxysmal and non paroxysmal focal dis turbances clear cut delta foci asymmetrical rhythmic activity and bilateral irregular spike and wave paroxysms In the present case all these findings could be traced at some stages of the disease The activity of the pathological process was most distinctly reflected by the disorganization and slowing down of the back ground activity in remissions the degree of disturbance was less than in relapses How ever the general trend of the EEG changes

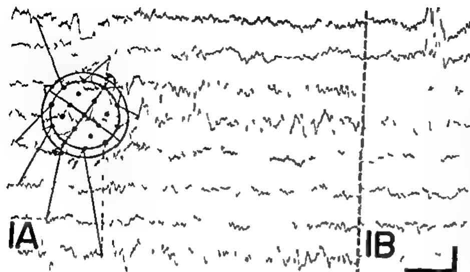


Fig 1 A and B EEG 9 2 1971 W organized alpha rhythm with do nating frequency of about 10 c/s *Moderately slow background* ac tivity in posterior derivations S spike and wave paroxysm oc curring spontaneously shown ir Fig 1 B Clinically patient in goo condition second remission Ca bration 1 s 100 uV The EEG montage in this figure is identica throughout

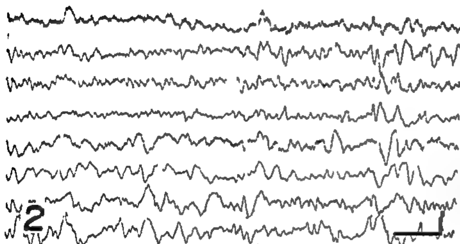


Fig 2 EEG 14 8 1974 Occipital rhythm slow and poorly organize Constant arrhythmic delta focus the left Abundant fast activity Clinically onset of relapse of 5–6 months duration No focal findin on neurological or radiological ex amination during this period

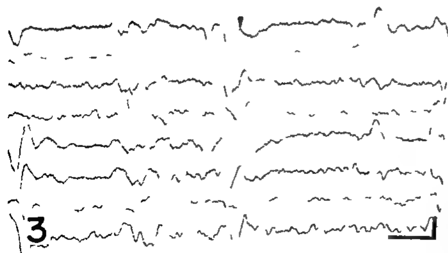


Fig 3 EEG 25 7 1975 Very slow disorganized EEG with frequent paroxysmal slowing and syn chronization no spikes Eyes open at arrow Clinically in a burned out state dies two months later





Fig. 4. The intracerebral blood vessels were thin walled without evidence of amyloid deposition not even in the area of the subacute intraparenchymatous haemorrhage in the right basal ganglia. Van Gieson stain. Bar 300 μm.

Fig. 5. Mainly mononuclear inflammatory infiltrate adherent to the wall of a vein in the midbrain with imminent perivascular spread. Van Gieson stain. Bar 100 μm.

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DISCUSSION

In a reprospective analysis of 100 children with JRA (4) different EEG abnormalities were common such as abundant beta activity, paroxysmal and non paroxysmal focal disturbances, clear cut delta foci, asymmetrical rhythmic activity and bilateral irregular spike and wave paroxysms. In the present case all these findings could be traced at some stages of the disease. The activity of the pathological process was most distinctly reflected by the disorganization and slowing down of the background activity; in remissions the degree of disturbance was less than in relapses. However, the general trend of the EEG changes

Table 1 *Essential EEG findings and their changes in seven consecutive records*

Age of patient on EEG examination	Dominating parieto occipital rhythm	Slow background activity	Fast background activity	Asymmetries and focal findings	Bilateral paroxysmal activity
10 y 1 mo	10 0 c/s well organized constant reaction to eye opening	Scanty to moderate concentrated to posterior regions	Scanty	No	Numerous theta bursts once a generalized relatively regular spike and theta wave discharge spontaneously
13 y 7 mo	8 8 c/s unregular poorly organized Variable and unconstant reaction to eye opening	Abundant with intensive episodic synchronization (2–3 c/s ad 300 μV)	Moderate	Intensive constant arrhythmic slow wave focus at left maximum temporally	Some generalized unregular sharp and slow wave discharges spontaneously
13 y 8 mo	6 8 c/s unchanged in organization and reactivity	Moderate with only moderate episodic synchronization	Abundant 18–20 c/s ad 50 μV rhythm concentreated frontally	Left side focus of less intensity and higher frequency Sharp transients at left	Many generalized or posterior polyspike and wave discharges only in flash light
13 y 10 mo	8 4 c/s better organized good reaction to eye opening	Moderate less synchronization than before	Abundant qualitatively unchanged	Abundant slow transients Constant rhythmic slow wave focus in right temporo-posterior region Sharp transients (esp in sleep) concentrated to right	Once a spike and wave discharge posteriorly in flash light
14 y 2 mo	7 8 c/s at times relatively well organized clear reaction to eye opening	Abundant episodic theta activity	Moderate	Abundant slow transients with changing asymmetries no constant focal finding	Many sharp and slow wave discharges of poor organization up to 4 sec in duration Mainly in drowsy state none in flash light
14 y 10 mo	7 6 c/s less well organized good reaction to eye opening	Increased in amount and intensity	Unchanged	Abundant concentrated at right but no constant focal findings	Unchanged
15 y 1 mo	5 2 c/s very poorly organized weak reaction to eye opening	Very intensive and abundant with frequent synchronization	Moderate to scanty mean frequency decreased	Changing asymmetries	No

was downward with a fluctuant course corresponding to the natural history of the disease

The most definite initial EEG abnormality consisted of bisynchronous spike and wave paroxysms which already occurred in the first EEG tracing recorded during the long lasting relapse (Fig 1A and B) Even though this abnormality need not have been caused by JRA (1 4) paroxysms got more and more disorganized and slowed during the course of the disease (Table 1) No spikes could be traced in the frequent delta paroxysms derived in the terminal stage (Fig 3)

Associated with a clinical relapse a grave hemispherical asymmetry was found which

initially appeared as a left sided arrhythmic delta focus and after its gradual disappearance as a more rhythmic right sided delta focus (Fig 2 and Table 1)

In spite of intensive clinical and neuroradiological investigations no focal findings could be verified except in the EEG Whatever the cause of the focal disturbances might be they as well as the simultaneous corticoid therapy may have resulted in psychotic symptoms which the patient had at that time During the observation time more and more both local and diffuse abnormalities remained in the EEG with a partially irritative character and changing morphology and localization In addition an abundant beta activity and fluctuating vigilance level without organized deeper sleep activity are characteristic of the EEG in the later stages of JRA

On microscopic examination no deposition of amyloid was found in intracranial vessels which agrees with the previous observations (6) Neither was there any direct evidence of cerebral vasculities However the presence of both an intracerebral haemorrhage and intra and perivascular inflammatory infiltrates corroborated the presumption of intracerebral vasculitis which has also been previously verified both angiographically (5) and histopathologically (7) in patients with RA

The assumption of transient toxic encephalopathy (3) does not appear probable because it hardly explains the grave hemispherical asymmetries and consistent residual findings in the EEG

To conclude JRA appears to be also a cerebral process with EEG findings broadly accompanying the natural history of JRA The main factors corroborating the aetiology of inflammatory vascular process in the present case are lacking cerebral amyloid fluctuant character of grave EEG asymmetries and the final cause of death—intracerebral haemorrhage without any vascular malformations (2)

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REVIEW ARTICLE

CLASSIFICATION OF INHERITED HYPO AND HYPERLIPIDEMIA

G E ANDERSEN

From the Neonatal Department Rigshospitalet Copenhagen Denmark

ABSTRACT Andersen G E (Neonatal Department Rigshospitalet Copenhagen Denmark) Classification of inherited hypo- and hyperlipidemia Acta Paediatr Scand 67 543 1978.—A model of lipoprotein dynamics is presented which permits a more rational understanding of the causes of the common types of inherited hypo- and hyperlipidemia in man with special reference to childhood

KEY WORDS Hypolipidemia hyperlipidemia genetics

Within the last few years many data have appeared which now enable us to assemble fragments of knowledge concerning lipids and lipoproteins into a dynamic system (1) The understanding of genetic lipid disorders is thus improved Hopefully better and safer treatment particularly urgent in the prevention of the premature vascular disease and CHD so often associated with these disorders will result

Most of these conditions can be diagnosed already in childhood Since the prevention of CHD is thought to be more efficient the earlier in life it starts pediatricians have become responsible for following the often very puzzling development in this exciting area of research In order to meet this need the following review is presented

Abetalipoproteinemia (ABL)
(Ia in Fig 1)

Frequency Very rare

Genetics Autosomal recessive Heterozygotes cannot normally be detected although in 4 kindreds (2) heterozygotes have been shown to have familial hypobetalipoproteinemia (see below)

Biochemistry Most likely there is a defect in Apo-lipoprotein B (apo B) synthesis (3) whereby the formation of TG carrying chylomicrons and VLDL is impaired

Blood findings Total lack of apo B chylomicrons VLDL and LDL Low levels of T C and TG

Clinical symptoms (*a*) Fat malabsorption because the lipid transport from gut to lymph is blocked MCT and linoleic acid may enter via the vena porta system The fat malabsorption leads without treatment to varying degrees of growth retardation (*b*) Retinitis pigmentosa and acanthocytosis, the cause of which is not known (*c*) Hypovitaminemia A and E Both vitamins are normally transported in LDL which is lacking in ABL (*d*) Neuropathy resembling Friedreich s ataxia with degeneration of long spino-cerebellar pathways and posterior and lateral spinal columns Although LDL seems to be required for optimal growth of CNS cells particularly during periods of rapid proliferation and myelination (4) there is some evidence that at least some of the

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Abbreviations

C=cholesterol C E=cholesterol ester T C=total cholesterol VLDL-C=very low density lipoprotein cholesterol LDL-C=low density lipoprotein cholesterol HDL-C=high density lipoprotein cholesterol TG=triglyceride CHD=coronary heart disease RES=reticulo-endothelial system MCT=medium chain triglycerides

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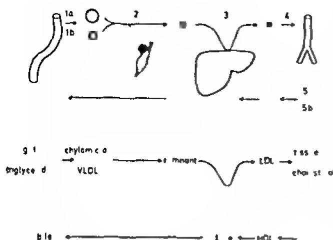


Fig 1 Model of lipoprotein dynamics

symptoms might be caused by lack of vitamin E in the cells (to prevent peroxidation of certain polyunsaturated fatty acids?) rather than by lack of LDL per se Several children with ABL have been (kept) more or less symptom free receiving high doses of vitamin E (5) (*e*) Although ABL patients have not yet reached the Western age for developing CHD there are suggestions that in ABL there is a remarkable lack of premature atherosclerosis and vascular disease but an increased risk of cardiac dysrhythmia the cause of which is not known

Differential diagnosis No other disease has total lack of apo B

Familial hypobetalipoproteinemia (1b in Fig 1)

Frequency Not known

Genetics Autosomal dominant (6) As pointed out above the parents of a few ABL children have been shown to have familial hypobetalipoproteinemia

Biochemistry Not known

Blood findings <5 percentile values for LDL and T C Normal VLDL and HDL This raises the question of what a normal concentration of LDL C should be Too high concentrations seen in homozygous familial hypercholesterolemia (see below) lead to severe atherosclerosis and CHD before age 20 Total lack of LDL seen in ABL leads to a variety of symptoms among which the CNS symptoms are the most serious In adult Western man serum LDL C concentration is about 5 times higher than in human newborns and in at least eight species of animals which do not develop atherosclerosis Also the serum LDL C concentration is 5 times higher than the concentration which has been calculated to saturate the non hepatic parenchymal cells need for hepatic LDL C (7)

To make the diagnosis of this condition demands a three vertical transmission within a family of LDL C concentration <5th percentile value corrected for age and sex which means that the diagnosis still is very troublesome in children because normal values of LDL C are not yet available for all ages Furthermore the influence of diet tends to obscure this condition particularly during the first year of life and in several cases it will not be possible to make a final diagnosis until after age 1 (8)

Clinical symptoms In a few individuals clinical pictures resembling Friedreich s ataxia and neurological symptoms involving demyelination of peripheral nerves have been described (9 10) but most individuals are totally healthy and may even be healthier than the average since they may be protected against atherosclerosis It is noteworthy that many of them obtain high or even very high age (85–100 years not being uncommon) Some of these individuals also seem to have a natural aversion to eating fatty meals and may even have a slight fat malabsorption (9)

Differential diagnosis Low levels of LDL are seen after trauma infections fat malabsorption hyperthyroidism hepatic necrosis severe anemia and anti apo B myeloma protein production

Familial lipoprotein lipase (LPL) deficiency (2 in Fig 1)

Frequency Not known

Genetics Autosomal recessive Homozy

gotes have low levels and heterozygotes slightly reduced levels of LPL

Biochemistry The first step of VLDL degradation involves a TG depletion by the action of LPL and a cofactor apo C II which leads to the formation of remnant particles (I II) (indicated by the dotted arrow in Fig 1 pointing towards adipose tissue around a muscle) There is an accumulation of chylomicrons and VLDL

Blood findings Highly elevated concentrations of chylomicrons and TG Overnight creamy top layer of plasma The LPL activity is measured indirectly by PHLA (post heparin lipolytic activity) Radioimmunoassays will soon be available since an antibody has been produced against LPL (12)

Symptoms Present from first meal in neonatal period Eruptive xanthomas on trunk lower extremities palate and tonsils lipemia retinalis After a large fatty meal acute pancreatitis sometimes leading to surgical intervention

Differential diagnosis Pancreatitis other conditions with elevated chylomicrons and TG e g FHTG (see below) In familial lipoprotein lipase deficiency there are no signs of diabetes nor abnormal glucose tolerance and no hypothyroidism

Broad beta disease (Remnant removal disease) (3 in Fig 1)

Frequency Not known Not normally diagnosed before age 20

Genetics Probably autosomal recessive (13)

Biochemistry The normal catabolism of VLDL to LDL is supposed to involve a TG depletion and C enrichment of VLDL particles by the action of LPL The resulting particles called remnants are probably catabolized to LDL particles by a hepatic triglyceride lipase (H TGL) and a cofactor apo E III acting together on the surface of the liver (indicated by the curved arrow in Fig 1) (14) The accumulation of remnants in broad beta disease is probably caused by a block in this last hepatic step (15) but the exact mechanism is not yet fully known

Blood findings The accumulation of VLDL like remnant particles (earlier known as floating pre beta particles) in the LDL density range 1 006–1 019 has given rise to the name broad beta disease T C and TG concentrations are elevated

Clinical symptoms Xanthomas typically in creases of palms but also tuberous and tendinous increased premature CHD and peripheral vascular atherosclerosis The reason for late penetrance (after age 15–20) is not known the balance between estrogens and androgens may play a role

Differential diagnosis Other conditions with elevated levels of T C and TG e g FH (see below)

Familial hypercholesterolemia (FH) (4 in Fig 1)

Frequency Homozygotes $1/10^6$ heterozygotes 1/500

Genetics Autosomal dominant Linkage to C 3 complement (16)

Biochemistry Hepatic LDL C is supplied to the extra hepatic parenchymal cells for membrane synthesis via high affinity LDL receptors so far demonstrated on the surface of cultivated fibroblasts (17) lymphocytes (18) and smooth aortic muscle cells (19) as well as freshly isolated lymphocytes (20) Surplus hepatic LDL C which is not cleared by these receptors is supposed to be catabolized by the so-called scavenger pathway (21) primarily the RES HDL is thought to play a role in carrying back free C from this system to a final breakdown in the liver to bile acids (22) The normal daily LDL degradation in adults has been calculated to be in the order of 1 000 mg of LDL C via the receptor pathway and 500 mg via the scavenger pathway In FH heterozygotes the LDL receptors are 50% reduced in number or function but since the amount of circulating LDL C is 2–3 times increased the total daily degradation of LDL C is in the order of 1 000 mg via the receptor and 1 000 mg

via the scavenger pathway In FH homozygotes no LDL clearance occurs and all degradation is via the scavenger pathway and averages 3000 mg of LDL C/day since in homozygotes the concentration of circulation LDL C is about 6–7 fold increased LDL C is toxic in such high concentrations and is probably trapped and precipitated by the glycosaminoglycans of the ground substance around the smooth muscle cells of the artery media This is thought to initiate the premature and severe atherosclerosis in FH patients

Blood findings Elevated LDL from birth In children aged 1–16 years the exact diagnosis may be impossible if the serum T C concentration is between 6 5–7 0 mmol/l because in this interval there is an overlapping of unaffected and FH heterozygotes (23) LDL C seems to be a better discriminator than T C (24) In the near future the exact diagnosis will probably be made possible by a combined measurement of serum LDL C and of the number or function of LDL receptors of e g lymphocytes in a child belonging to a family with three vertical transmission of hypercholesterolemia or xanthomatosis

Clinical symptoms Cholesterol deposition in the form of planar and tuberous xanthomas and xanthelasmas Premature and severe CHD which without treatment leads to death before age 20 in homozygotes In heterozygotes CHD symptoms in males occur already in early or mid adult life In females the risk is not as great as in males but still considerably greater than in normal females (25)

Differential diagnosis Elevated LDL C can be found in nephrosis hypothyroidism biliary cirrhosis diabetes dysglobulinemia porphyria and after steroid treatment

Familial hypo alphalipoproteinemia (Tangier disease) (5a in Fig 1)

Frequency rare

Genetics Autosomal recessive

Biochemistry Synthesis of an abnormal HDL

Blood findings Lack of normal A I apoprotein (26) Low HDL levels (<5th percentile values)

Clinical symptoms CE accumulation in macrophages of the RES leading to yellow enlarged tonsils and hepatosplenomegaly Peripheral sensory neuropathy The risk of developing premature vascular disease is not known

Differential diagnosis none

Familial hyper alphalipoproteinemia (5b in Fig 1)

Frequency Not known

Genetics Autosomal dominant (27)

Biochemistry Not known

Blood findings >95 percentile values for HDL C in three generations Elevated or normal T C depending on a concomitant low or normal concentration of LDL C

Clinical symptoms Not a disease but a condition in which the high HDL levels probably protect against atherosclerosis leading to longevity

Differential diagnosis Elevated HDL levels may be seen in biliary cirrhosis alcoholism and after exposure to chlorinated hydrocarbon pesticides and estrogen supplementation

Other types of hyperlipoproteinemia which cannot be explained with our present knowledge of lipoprotein dynamics are

Familial hypertriglyceridemia (FHTG) and *Familial combined hyperlipemia (FCHL)* These conditions do not normally become manifest before age 20 and there is still much doubt whether or not they are true monogenic disorders (28) The first may become manifest in children during untreated diabetes mellitus The latter owes its name to the fact that one third of the patients has elevated T C one third has elevated TG and one third has a combined elevation of both serumlipids

Polygenic hypercholesterolemia responsible for the upper 3–4% of the normal cholesterol distribution (Not explained by the above mentioned conditions with high T C levels) There seems to be a certain correla

tion between high values of T C in parents–offspring and siblings twins especially but not between spouses There is a higher risk than normal of premature CHD in these individuals

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NEW BOOKS RECEIVED

S Saint Anne Dargassies (ed) *Neurological development of the full term and premature neonate* 373 pp illus Excerpta Medica Amsterdam London New York US $68 95 ISBN 90-219 2109 X

F Falkner (ed) *Fundamentals of mortality risks during the perinatal period and infancy* 208 pp illus *In* Monographs in Paediatrics ■ Karger Basel Munchen Paris London New York Sydney 1977 Vol 9 DM 24 – ISBN 3-8055 651 2

R Mande N P Masse & Manciaux (eds) *Pediatrie sociale* 2nd ed 708 pp Flammarion Medecine Sciences Paris 1977 No price given ISBN 2 257 20381 X

G Schön & U Heller Schon (eds) *Molekularbiologie und klinische Bedeutung des Stoffwechsels normaler und modifizierter Nucleobasen* 177 pp Schwabe & Co Verlag Basel/Stuttgart 1977 Fr /DM 36 – ISBN 3 7965-0683 8

Toward a primary medical care system responsive to children s needs Harvard Child Health Project Report vol 1 83 pp Ballinger Publishing Company Cambridge USA 1977 £8 70 US £14 30 ISBN 0-88410-507 5

J L Melnick (ed) *Progress in medical virology* vol 23 212 pp S Karger Basel Munchen Paris London New York Sydney 1977 US $44 – ISBN 3-8055 2423-4

David J Hall *Social relations and innovation* 222 pp *In* Medicine illness and society Routledge & Kegan Paul London Henley and Boston 1977 £5 25 ISBN 0-7100-8607 5

Richard E Behrman (ed) *Neonatal perinatal medicine Diseases of the fetus and infant* 1010 pp illus The C V Mosby Company St Louis Missouri 1977 US $49 50 ISBN 0-8016-0579 2

H J Kaufmann (ed) *Progress in pediatric radiology* vol 6 Skull spine and contents part II Clinical aspects 304 pp illus S Karger Basel Munchen Paris London New York Sydney 1978 US $49 75 ISBN 3 8055 2337 8

Morris Green & Robert J Haggerty (eds) *Personal health care of children in the office* 500 pp illus *In* Ambulatory pediatrics II W B Saunders Company Philadelphia London Toronto 1977 No price given ISBN 0-7216-4236-5

R Angus Harkness & Forrester Cockburn (eds) *The cultured cell and inherited metabolic disease* 203 pp illus International Medical Publishers Lancaster 1977 £10 95 ISBN 085200-167 3

Jack G Shiller *Childhood injury* 256 pp illus Stein and Day Scarborough House Briarcliff Manor N Y 10510 1977 No price given ISBN 0-8128-2066-5

Harry C Shirkey *Pediatric drug handbook* 198 pp W B Saunders Company Philadelphia London Toronto 1977 No price given ISBN 0-7216-8247 ,

J C Somogyi (ed) *Nutritional psychological and social aspects of obesity* 158 pp illus Bibliotheca Nutritio ■ Dieta No 26 S Karger Basel Munchen Paris London New York Sydney 1978 DM 85 – US $38 – ISBN 3 8055 2764-0

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BOOK REVIEWS

Marvin Cornblath & Robert Schwartz *Disorders of carbohydrate metabolism in infancy* 2nd ed *In* A J Schaffer & M Markowitz (eds) Major Problems in Clinical Pediatrics Vol III 501 pp illus W B Saunders Company Philadelphia London Toronto 1976 No price given ISBN 0-7216-2721 8

When the first edition of this book appeared in 1966 it soon became a standard book of reference on carbohydrate metabolism in infancy Now the second edition which is updated and expanded has arrived

The first chapter on The Metabolism of Carbohydrate has been greatly expanded with a review of developmental biochemistry by Alan L Schwartz son of one of the authors This is an excellent review of the present knowledge in the field

The rest of the book is very clinically orientated with an impressive reference list following each chapter There is a clear account in each chapter of the physiological basis from which the authors draw their conclusions as to therapeutic measures Their reviews on clinical manifestations diagnostic procedures management and prognosis for different disease entities are notably clear and easy to follow The book is of an even high standard throughout but Chapter 5 Hypoglycemia in the Neonate is particularly worth reading since it bears the mark of the authors long standing interest and wellknown investigations in the field

In Appendices I and II very useful tables concerning Diets for disorders of carbohydrate metabolism and Carbohydrate content of foods are included

In short this excellent book is warmly recommended to everyone interested in neonatology and in particular to those interested in disorders of carbohydrate metabolism in infancy Furthermore this monograph ought to be considered a must in the library of every pediatric clinic

Johan Gent.

Ciba Foundation Symposium 45 *Breast feeding and the mother* 280 pp illus Excerpta Medica Amsterdam 1977 US$20 95 (clothbound) $11 50 (paperback) ISBN 90-219-4051 5

The decline in breast feeding in many societies has become a subject of major concern to health workers throughout the world It is not surprising then that breast feeding has been discussed in three recent CIBA Foundation symposia Parent–infant interaction (1974) Acute diarrhoea in childhood (1975) and Breast feeding and the mother (1976) The last symposium is now published (18 contributing papers 280 pages)

The first seven papers deal mainly with various aspects of the physiology of lactation including the production and role of prolactin (the hormone of love as it has been termed involved as it is in the various stages of reproduction) The remaining eleven papers take up major topics such as The Importance of Early Mother-Infant Contact Cross Cultural Aspects of Breast Feeding Community and Socio-Political Considerations of Breast Feeding and so forth Each paper is followed by long discussions providing further valuable information

Anyone interested in lactation soon realizes that the topic is vast and that it is central in human physiology and psychology There is a danger in trying to cover all of this simultaneously Some of the contributions on hormonal experiments in animals are too highly specialized to fit in with the remaining more central aspects in this non textbook volume

On the whole however the symposium has a very high standard Breast feeding is sparsely dealt with in most undergraduate textbooks in paediatrics and obstetrics Published symposia of this type are therefore welcome and the present one is highly recommended for reading by all involved There are a total of 553 references listed!

Yngve Hofvander

J H Emans & D P Goldstein *Pediatric and adolescent gynecology* 195 pp illus Little Brown and Company Boston 1977 No price given ISBN 0-316-23400-1

The textbook oriented to pediatricians family practitioners students and nurses is a simplified approach to the common gynecological problems of the child and the adolescent The first chapter of the book is a clinical guide to the most common gynecological examinations of the child or adolescent with particular attention to the emotional aspects In a separate chapter the physiology of puberty is briefly reviewed as a background to problems of delayed development and menstrual irregularities A special interest is focused to vulvovaginal problems both in the prepubertal child and in the adolescent The treatment regimens differ on some points from Scandinavian routines—for example estrogen creams applied locally are seldom used in children to get the vaginal mucosa acidic—but the authors intentions are only to suggest different ways of therapy

Dysmenorrhea is the most common gynecological complaint of the adolescent Although the etiology is unclear prostaglandins may play an important role in stimulating contractility of the myometrium which explains the good therapeutic effect of antiprostaglandin drugs as indomethacin and flufenamic acid These drugs are unfortunately not registrated in the Scandinavian countries on this indication and therefore oral contraceptive pills have been the dominating therapeutic drugs for dysmenorrhea Problems concerning birth control are briefly discussed and the efficacy and side effects of the various methods

treated in great detail The authors lay stress upon the fact that the teenager must participate in the decision on what form of contraception is best for her Only a motivated adolescent uses a prescribed contraceptive method

The chapters concerning teenage pregnancy and rape contains much good advice on how to handle the patient and the parents in difficult situations and the textbook ends with some general aspects on sex education

A great number of illustrations tables and references increase the value of the book which can be highly recommended to the categories of readers mentioned

Lars Svanberg

J G Wilson & F C Fraser (eds) *Handbook of teratology* vol 1 General Principles and Etiology 476 pp illus Plenum Press New York and London 1977 US $34 00 ISBN0-306-35 41-4

This is the first of four volumes of a handbook written according to the Introduction for graduates and other professional students and beginning scientists It is prepared by highly qualified scientists and covers various basic fields in teratology Josef Warkany introduces with a fascinating history of teratology describing ancient reproductions of monstrosities quoting writings from various periods of human history and describing the changing attitudes of society towards the abnormal baby and its parents Wilson introduces with Current Status of Teratology where among other things various possible mechanisms of teratogenesis are discussed However the central and intriguing problem of embryonic induction is ignored The second editor Fraser has an introductory chapter of high quality on the relation of animal studies to the problem in man

The second section discusses various causes of malformations mutagens radiation infectious disease nutritional deficiency drugs and other chemicals each chapter written by a specialist in the field The contributions are mainly of high quality One can always question the selection of information included and the evaluations made particularly perhaps in Wilson s chapter on drugs in human teratogenesis It does not make full use of available information from epidemiological studies instead it quotes much animal data with sometimes doubtful relevance This area is rapidly expanding and some of the information given is already outdated Wilson also contributes a chapter on environmental chemicals again mainly based on animal experiments His conclusion is in view of the limited observations summarized in this chapter it can hardly be maintained that any aspect of human development is seriously threatened at this time by chemical agents in the environment This chapter also includes a short and sketchy discussion on the possible effect of smoking perhaps the quantitatively most important environmental hazard to the developing human organism

The over all impression of this volume is that of a learned work the broad outlines of which fulfil the editors intentions but should be read with some caution We look forward to the following volumes of the handbook which will cover among other subjects epidemiological aspects

Bengt Kallen

Craig Oman *Childhood diabetes and its management* 165 pp illus *In* (ed John Apley) Postgraduate Paediatrics Series Butterworths London and Boston 1977 £8 50 ISBN 0-407-00176-3

In the foreword John Apley Editor of the Postgraduate Series writes that he cannot remember a medical book that has charmed him more and given him greater enjoyment in the reading The author really knows how to express himself in a lively brilliant way and it is evident that he has a wide clinical experience and a thorough practical and theoretical knowledge in the field He describes insulin and metabolism in a simple way and the discussion on ketoacidosis and the treatment of coma is very instructive The chapters on the course of childhood diabetes includes several interesting remarks about remission although some recent results from studies on beta cell function are lacking This may explain why the author does not believe that the remission is a consequence of improved insulin secretion In the chapter on Complications the author describes skin lesions oedema and neuropathia but he means that late vascular complications of adult life is beyond the scope of this small book

The management of diabetes is controversial and it would be remarkable if every recommendation in the book could be accepted without discussion The author writes There was a time when it was thought that deliberate overtreatment in hospital for the first few weeks improved the longterm prognosis I doubt if many holds this view At the same time he notices that there may be a tendency for insulin requirements to rise in the first few days after onset Perhaps this would not occur if the treatment were more intensive from the beginning? — In contrast to the view of many Scandinavian paediatricians the author usually prescribes only one daily dose of insulin and he passes over his diabetic patients to the adult physicians already at the age of 13–15 years He means that the most important factor in producing good control is an inherent tendency for the diabetic individual to stay stable and he points to that many believe that the end results of fair control are as satisfactory as those of good control" However there seems to be overwhelming evidence showing that late vascular complications are a consequence of metabolic disturbances and it seems to me too nihilistic to accept an inherent tendency as the most important factor in fluencing the degree of control

Although opinions differ in some questions the author gives his views in a reasonable way without being dogmatic He discusses strictness liberality and control and concludes that the scales will be tilted from the liberal side but I am not convinced that all scandinavian paediatricians agree when the author describes two daily urine tests as the liberal view Obviously he is correct when he emphasizes that the difference between strictness and liberality is not simply that between black and white

This is a book not just about biochemics or syringes It is about children and their families The book includes two instructive chapters about the psychological problems behaviour and communication The author writes It has often been said that diabetics should be told they

can lead a completely normal life I think this is wrong particularly with children The child knows perfectly well he is not leading a normal life Since when were injections urine testing diet and strict time keeping normal for a child? The author means that the paediatrician should be concentrating on promoting in the child an attitude which is honest accepting and responsible

The book is permeated with this attitude and the author s sympathy for the diabetic children Indeed this is a stimulating book that can be recommended to all paediatricians and other physicians dealing with juvenile diabetics and I agree with John Apley in his last sentence in the foreword I hope other readers will get as much practical help wisdom and sheer enjoyment from it as I have

Johnny Ludvigsson

Clement A Smith & Nicholas M Nelson (eds) *The physiology of the newborn infant* 4th ed 771 pp illus Charles C Thomas Publishers Springfield Ill 1976 US $52 00 ISBN 0-398 03232 7

Clement A Smith s book on the physiology of the newborn infant for a long time held the undisputed place as first hand source of information and inspiration for paediatricians with a special interest in neonatal care The fourth edition of this work has now appeared 17 years after the preceding one In the new edition the editorship is shared between Dr Smith and Dr Nicholas M Nelson The book has grown markedly from 400 one column pages in the third edition to 750 two-column pages in the present one Thus it has turned into the heavy reference type of book It has lost some of its distinctly personal tone of voice from previous editions but the book has gained not only in size but also very much so in contents The chapters on circulation and respiration have developed into very comprehensive reviews with altogether 944 references This overwhelming number of references to original work had however been still more useful had they been listed in an alphabetical order instead of order of appearance in the text

The book also covers kidney physiology bilirubin metabolism digestion and nutrition as well as fetal and neonatal endocrinology with excellent and extensive reviews

With a book of this size and with eleven contributors a long production time is probably unavoidable Some chapters were apparently finished considerably earlier than others—or in Dr Smith s wording Much the greatest disadvantage to all such books are the surprising differences in the rate of literary composition Therefore the author of the chapters should probably not be blamed for omitting such new and exciting discoveries as fetal breathing movements or the importance of the renin–angiotensin system for maintenance of fetal and neonatal blood volume or the role of prostaglandins in the state of contraction of ductus arteriosus

This book should be a most useful textbook for neonatologists and physiologists Its rapid rate of growth should be taken as a sign of viability We look forward to the next edition in less than 17 years

Ingemar Kjellmer

ANNOUNCEMENT

ASSOCIATION FOR PEDIATRIC EDUCATION IN EUROPE

The Annual Meeting of the Association for Pediatric Education in Europe will be held in Cracow/Warsaw (Poland) during the period September 18–21 1978 The main topic will be *Teaching communication with children* Requests for information should be sent to Professor Z Kobielowa M D Head of the 1st Pediatric Clinic Pediatric Institute of University Medical School Nicolaus Copernicus Wielicka 265 *30-663 Cracow* Poland

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BREAST FEEDING AND COMPOSITION OF HUMAN MILK— RECENT PROGRESS IN OUR KNOWLEDGE

Symposium held at the XVth International Pediatric Congress N Delhi 24th October 1977

Guest editors P ROYER Paris and B VAHLQUIST [1] Uppsala

The following is a presentation of the introductory papers at the Symposium The motto of the N Delhi Congress was Breast feeding with love leads to better child health This is beautiful wording which has been given added strength by the underlining of the paramount importance of breast feeding in other Symposia and Communications also as well as in the concluding Plenary session on The challenge of global malnutrition Or to quote from the address by Professor C Gopalan Director General of the Indian Council of Medical Research the remarkable ability of poor women to breast feed their babies for prolonged periods is the most redeeming feature in an otherwise bleak nutritional situation of many developing countries

The 1970 s have witnessed a strong upsurge of interest in breast feeding in many industrialized countries At the same time considerable knowledge has been gained both with respect to breast feeding patterns in different parts of the world and to chemical and immunological properties of human milk

This Symposium focuses on recent advances in specific important areas rather than on a broad overview The authors are all thoroughly familiar with the fields which they have agreed to discuss The field data on the frequency and duration of breast feeding in different social groups as well as on the duration of lactational amenorrhoea in the same groups represent a limited selection of information from a forthcoming WHO/CIE Report on Collaborative study on breast feeding Out of a total of nine countries co-operating three form the basis for the data given in this context We want to express special thanks to WHO/CIE and to the principal investigators involved (Guatemala Dr J J Urrutia the Philippines Dr G Guzman and Sweden Drs E Sjolin and Y Hofvander) for their permission to include the relevant information

P Royer B Vahlquist

[1] Professor Bo Vahlquist died March 31 1978

BREAST FEEDING AND BIOLOGICAL DEVELOPMENT

P ROYER

From the Hopital des Enfants Malades Paris France

ABSTRACT Royer P (Hopital des Enfants Malades Paris France) Breast feeding and biological development Acta Paediatr Scand 67 554 1978 —The superiority of human milk as compared with milk of other origin for the feeding of newborns term or preterm can be analysed in terms of biological development related to digestive metabolic and excretory functions during foetal and postnatal life The macro- and micro-anatomical developments of the intestine are complete in the 6th foetal month The brush border and some of its enzymes (saccharase isomaltase) exist already from the 6th foetal week whereas other enzymes (lactase and intracellular transport enzymes) appear much later The major gastric and pancreatic enzymes as well as the synthesis of biliary acids do not reach maturity until after birth Several metabolic functions e g the synthesis of cystine from methionine of tyrosine from phenylalanine and of urea from ammonia are still limited at the time of birth The capacity for excretion of sodium the osmotic urinary load and hydrogen ions is suboptimal especially in the prematurely born All these circumstances imply that human milk with its protective properties represents optimal adaptation to the needs of the child in the perinatal period

KEY WORDS Mammary gland development foetal

The development of the human mammary glands begins in the 7 mm long embryo in the form of faintly outlined bands arranged in pairs The caudal pair soon disappears leaving one pair After birth there is a brief swelling of the mammary glands which may be accompanied by secretion of a milk like substance The final development in the female occurs at puberty and goes through five stages which may be identified and observed on superficial examination It is clear that the development of the mammary glands depends on sex steroids—oestrogens and progesterone—and that the milky secretion is regulated by prolactin Through the course of animal evolution this hormone has played very different roles It is interesting to note that several of its actions *facilitate* the reproductive developmental cycle for example its action on the kidneys of migrating teleost fishes its effect on the parental behaviour of birds amphibians and certain fish and its provocation of secretions that are specific for the nutrition of the young—the milk of the cutaneous glands of certain fish the secretion of the crop in pigeons and the milk in mammals

The superiority of breast milk over other kinds of milk as food for the human newborn whether premature or full term should preferably be analysed within the context of the progressive development of digestive metabolic and excretory functions during gestation and after birth

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In contrast with intestinal activity the de velopment of gastric functions does not end at birth when the acid secretion is low It almost reaches an adult level at 3 months of age and is mature at 24 months the levels of pepsin se cretion follow the same pattern and the in trinsic factor secretion becomes maximal 3 months after birth (5) The lobar and lobular structure and the acini and canaliculi of the pancreas are laid down at 16 weeks and the zymogen granules in the 20th week of foetal life but at birth the α amylase (Amy 2) ac tivity is almost zero and the lipase trypsin and chymotrypsin activities are low and con tinue to increase up to the age of 2 to 3 years The rate of digestion of casein is almost iden tical in infants (1 g/kg/hour) and adults (1 6 g/kg/hour) Biliary acid synthesis is noted after the 15th foetal week but still remains too low both in premature and full term newborns to reach adequate micellary concentration levels (9)

2 An important aspect in the *metabolic adaptability* to breast milk is the perinatal and postnatal development of the enzymatic systems of interconversion and elimination The low level of cystathionase activity limits the cystine synthesis based on methionine (4) At birth the cystine is thus an essential amino acid breast milk which contains one and a half to twice as much cystine as cow s milk is better adapted It is possible that tyrosine syn thesis based on phenylalanine is also limited On the other hand the enzymes of the urea cycle are present before the 12th week of foetal life but the activity of the entire system remains limited at birth and the maximum quantity of urea synthesized by full term in fants corresponds to an intake of 2 5 to 3 g/kg of protein (7)

3 The development of *kidney function* is not complete at birth For the past few years it has been clear that in this respect breast milk is better adapted to renal regulatory functions after birth (1) such as the control of sodium excretion excretion of the osmotic urinary load and excretion of hydrogen ions and regu lation of the acid base equilibrium These re gulatory mechanisms are increased in the premature infant Humanized milks provide partial solutions to this problem (8)

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For these reasons human breast milk would seem to present the optimal qualities per mitting the newborn to adapt to his new en vironment

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BREAST FEEDING AND BIOLOGICAL DEVELOPMENT

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KEY WORDS Mammary gland development foetal

The development of the human mammary glands begins in the 7 mm long embryo in the form of faintly outlined bands arranged in pairs The caudal pair soon disappears leaving one pair After birth there is a brief swelling of the mammary glands which may be accompanied by secretion of a milk like substance The final development in the female occurs at puberty and goes through five stages which may be identified and observed on superficial examination It is clear that the development of the mammary glands depends on sex steroids—oestrogens and progesterone—and that the milky secretion is regulated by prolactin Through the course of animal evolution this hormone has played very different roles It is interesting to note that several of its actions facilitate the reproductive developmental cycle for example its action on the kidneys of migrating teleost fishes its effect on the parental behaviour of birds amphibians and certain fish and its provocation of secretions that are specific for the nutrition of the young—the milk of the cutaneous glands of certain fish the secretion of the crop in pigeons and th milk in mammals

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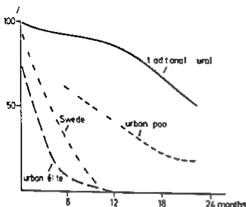


Fig 1 Breast feeding—Guatemala WHO collaborative study 1976

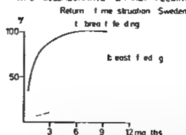


Fig 3 Return of menstruation in breast feeding and not breast feeding mothers in Sweden (urban and rural) WHO collaborative study 1976

different countries to study the problem and to formulate programmes of intervention The WHO Task Force has gone on to select a number of areas for study These are

- The frequency and duration of breast feeding and factors influencing these parameters —a basic study
- The quantity of breast milk consumed at various periods after birth
- Breast milk quality its main constituents as well as trace metals immunoglobulins pesticides and other environmental elements
- The anti-conceptional value of breast feeding
- Legislation health service and training in relation to breast feeding
- The production and marketing of baby foods in particular breast milk substitutes
- Action/intervention programme

The basic study

The basic study was started in 1975 and concentrated on the epidemiology of breast feeding among three different socio-economic groups namely the urban elite urban poor and traditional rural Nine countries (Chile Ethiopia Guatemala Hungary India Nigeria Philippines Sweden Zaire) participated in the study and altogether almost 24 000 mother/child pairs were involved The study was conducted on the basis of a questionnaire interview The questionnaire had previously been tested in four countries (Guatemala Ethiopia India and Sweden) and a number of local adaptations were made

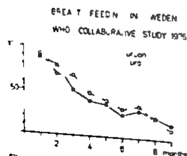


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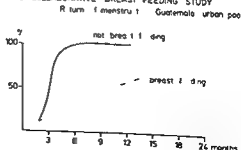


Fig 4 Return of menstruation in breast feeding and not breast feeding mothers in Guatemala (urban poor) WHO collaborative study 1976

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WHO COLLABORATIVE STUDY ON BREAST FEEDING

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ABSTRACT Hofvander Y and Petros Barvazian A (Department of Paediatrics University Hospital Uppsala Sweden and Division of Family Health World Health Organization Geneva Switzerland) WHO Collaborative Study on Breast Feeding Acta Paediatr Scand 67 556 1978 —WHO concerned with the declining breast feeding rate in developing countries has organized investigations in nine different countries of different aspects of breast feeding and breast milk with the ultimate aim of formulating programmes of intervention The basic epidemiological study on 24 000 mother/child pairs is just completed and some preliminary data are reported on the pattern of breast feeding in the three studied socio-economic groups—urban elite, *urban poor and traditional rural—which differ significantly* in their breast feeding rate as well as in the pattern of return of menstruation in breast feeding and non breast feeding mothers

KEY WORDS Breast feeding rate socio-economic groups developing countries

BACKGROUND

There has been a significant decline in the incidence and duration of breast feeding in many industrialized countries This decline has been particularly marked in the United States where now only a small percentage of mothers are found to be breast feeding on discharge from maternity hospitals A similar trend has been observed in European countries although during the last 3–5 years there has been evidence of a reversal among some socio economic groups (4)

Observers have pointed to corresponding trends in developing countries particularly among urban populations The implications of this for the nutritional condition of young infants in developing countries are serious

The World Health Organization concerned with this development has taken the initiative to bring together a *Task Force of experts from*

Table 1 *Number of antenatal visits—Guatemala*

WHO collaborative study on breast feeding

	■	1–4	4–9	10
Urban elite	–	1	13	86
Urban poor	8	16	51	5
Rural	34	9	35	2

population than in the rural group Whether diet lifestyle or health status are critically related to the return of menstruation in these groups is not clear but the question deserves to be followed up

Pattern of breast feeding

The breast feeding development in Sweden is interesting as an example of what is apparently happening in certain western industrialized countries The development since the 1940s indicates a progressive decline up to about 1972 (7) (Fig 5) During the last few years there has been a definite increase At 2 months in 1972 31% were completely breast fed while the figure in 1975 was 46% At 6 months the corresponding figures were 6 and 14% respectively i e a 2½ times higher rate in just a few years From earlier studies we know that the highest breast feeding rate is found among women more than 25 years old who live in a stable marriage or co-habitation and who are well educated (6) This is obvious from Fig 6 Fifty six per cent of those with university education were breast feeding at 6 months 33% of those with secondary education and 22% of those with primary education All through the breast feeding period the difference was well noticeable

If we compare these figures with a few from the Guatemalan data we can see that there are marked differences (Fig 1) The Guatemalan urban elite for example breast feeds far less than does the Swedish elite And if we consider the pattern among the urban poor of Guatemala and relate this to education an opposite trend to that observed in Sweden again emerges—the higher the education the lower the rate of breast feeding (Fig 7)

A number of additional factors are being examined Maternal age the sex of the child and the length of time the mother has lived in her present place of residence are all being considered as possible factors related to breast feeding practice So far however these factors do not seem to be influential within the given socio-economic groups And although the number of antenatal visits is considerably higher among the urban elite population than among the other two socio economic groups of Guatemala it is again not yet clear to what extent this will be related to breast feeding practice (Table 1)

In Guatemala 89% of the urban poor population were delivered in hospitals in the rural area the figure was 13% Only 6% of all mothers delivered in hospitals started breast feeding within 12 hours of delivery while of those who were delivered at home 30% established breast feeding within the first 12 hours Approximately 20% of the urban poor mothers who were delivered in hospital were provided with free milk samples and in another 10% free feeding bottles were given The data suggest that hospital routines and the value system reflected in those routines may be an influential factor in determining infant feeding practices

ACTION PROGRAMME

The WHO Collaborative Study on Breast Feeding was initiated with the overall aim of achieving a better understanding of the various factors that influence breast feeding patterns in different settings and not for the purpose of proving the obvious value of breast feeding The idea was that once the various factors influencing breast feeding patterns are better understood the specific action and intervention programme suitable to each country and setting could be initiated to improve infant nutrition The action programme thus developed would be more effective and efficient

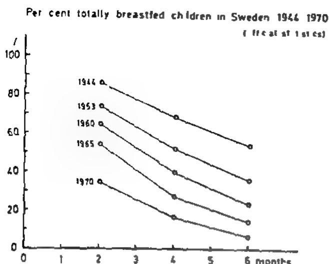


Fig 5 Per cent totally breastfed children in Sweden 1944–1970 (official statistics)

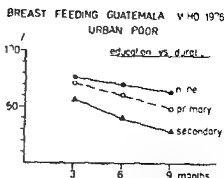


Fig 7 Breast feeding education vs duration—Guatemala WHO collaborative study 1976

Compilation

Data from all nine countries have now been compiled by the principal investigators and sent to WHO/HQ in Geneva There they are being centrally analysed and a report reflecting the findings of this study will appear late in 1978 The report will contain not only an overview of the results but also more detailed reports which will highlight internal variations within each country Results of a few of the important *preliminary* findings are presented here

RESULTS

Duration of breast feeding

Although the analysis is at a very primary stage some trends are already observable As far as the duration of breast feeding is concerned significant differences are appearing between the three different socio economic groups studied in each country In most settings it is apparent that breast feeding declines most rapidly among the urban elite group and is most prolonged in the rural traditional populations (Fig 1) [1] In the case of Sweden however where the population is more homogeneous both socio economically and in terms of health background the differences between urban and rural groups are not marked (Fig 2)

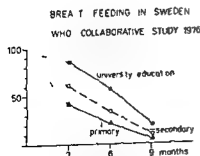


Fig 6 Breast feeding vs education—Sweden WHO collaborative study 1976

Return of menstruation

It has long been held that post partum amenorrhoea is more prolonged in breast feeding than in non breast feeding mothers The data so far collected and analysed corroborate this clearly (Figs 3 and 4) This phenomenon however is complex Comparing the situation among the urban poor and rural mothers of Guatemala there appears to be a much earlier return of menstruation among the urban population irrespective of breast feeding patterns Thus two months after delivery 16 7% of the urban mothers who were still following a schedule of complete breast feeding reported a return of menstruation Among a comparable rural traditional population on the other hand the percentage was only 2 4% Differences continue to emerge throughout the first 21+ months and at all age intervals post partum menstruation occurs earlier among the urban

[1] Figs 1 2 3 4 6 7 8 and Table 1 are based on references 1 2 3 8

NITROGEN AND PROTEIN COMPONENTS OF HUMAN MILK

L. HAMBRÆUS, B. LÖNNERDAL, E. FORSUM and M. GEBRE-MEDHIN

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ABSTRACT. Hambraeus, L., Lonnerdal, B., Forsum, E. and Gebre-Medhin, M. (Institute of Nutrition, University of Uppsala, Uppsala, Sweden). Nitrogen and protein components of human milk. Acta Paediatr Scand, 67: 561, 1978.—The true protein content of human milk is 0.9% in well nourished as well as malnourished mothers. Casein constitutes only about 20% of the protein nitrogen in human milk. The remaining 80% is derived from the whey proteins, the three dominant components being α-lactalbumin, lactoferrin and secretory IgA. α-lactalbumin is a subunit of lactose synthetase. Lactoferrin is an iron binding glycoprotein which plays a role in the defence against gastro-intestinal infections and it probably also involved in iron transport in the gut. Secretory IgA is comparatively stable at low pH. It is resistant to proteolytic enzymes and plays an essential role in the immunological defence against gastro-intestinal infections. Lysozyme is a minor component of the whey proteins and represents an active enzyme with a bactericidal effect. The nutritional and immunological significance of the marked differences with respect to the nitrogen and protein compositions of human milk and cow's milk should not be underestimated, but need further elucidation.

KEY WORDS: Human milk, protein components, infant nutrition

Our knowledge concerning the nitrogen and protein compositions of human milk and their variation has long been very limited. Many years ago Macy and her collaborators performed a series of outstanding studies on the composition of human milk (12, 13). At that time, however, the only available methods for qualitative and quantitative analysis of milk were those which were originally developed for the analysis of cow's milk. They were therefore not always applicable to human milk, as there are physico-chemical differences between bovine and human casein which lead to different physico-chemical characteristics and subsequently different curd formation (15). This is of nutritional interest, since the curd formation influences the digestion of protein by the gastric enzymes in the stomach of the young infant.

On the other hand, certain findings by Macy and her group, e.g. the high content of non-protein nitrogen, have oddly enough been overlooked and not taken seriously into account. Thus it has been wrongly assumed that the protein content in human milk is 1.1 to 1.2%, which represents an overestimation by 20 to 25% (8). The observation that 20–25% of the total nitrogen is in the form of non-protein nitrogen has since been verified in our studies on larger materials on well nourished as well as in malnourished mothers (9, 10).

Table 1 shows the nitrogen composition of human milk—both colostrum and mature milk. The data given in this table were obtained in a study on milk specimens from well nourished mothers, in which a complete analysis of the milk composition was performed (11). It is seen that non-protein nitrogen constitutes about 13% of the total nitrogen in colostrum but, as said before, about 25% in mature milk. The main part of the non-protein nitrogen is derived from urea, 11% and 33% respectively.

Table 1. *Nitrogen composition of human milk*
The values refer to mg N per ml

	Colostrum	Mature milk
Total nitrogen	3.60	1.71±0.31
Protein nitrogen	3.13	1.29±0.26
Non-protein nitrogen	0.47	0.42±0.10
Urea nitrogen	0.05	0.14±0.03

since it would be addressed specifically to the factors influencing breast feeding and infant feeding in a given area The objective of the studies is thus to stimulate the development of action programmes at the national level designed to improve better infant feeding A number of factors will need to be taken into account and many of these have already been identified as part of the study results

Lifestyle educational background and the ecological conditions in which the different groups live are all important The reasons reported by the mother why she did or did not breast feed or why she did not introduce supplementary feeding at a particular time vary considerably from setting to setting Nevertheless there appears to be a common theme running through many of the responses Answers such as the milk had dried up or the child was hungry occur repeatedly Although there can be little doubt that in many cases the mother may reach a maximum milk production capacity at about 500–600 ml (5) the easy availability of breast milk substitutes may have created a diminished confidence in her own ability to produce sufficient milk to meet the infant s needs Breast feeding is a confidence trick (4)

There are also indications that the advice and influence of health personnel may have played a part in the promotion of early supplementation and weaning Similarly the fact that in developing countries a higher educated group tends to breast feed least of all suggests that education cum lifestyle is an important predisposing factor At the same time the current trend observed in Sweden among better educated mothers suggests that as appropriate information is provided and internalized by mothers breast feeding practices will improve and that over time similar trends may emerge in what are now developing countries

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ABSTRACT. Hambræus, L., Lonnerdal, B., Forsum, E. and Gebre-Medhin, M. (Institute of Nutrition, University of Uppsala, Uppsala, Sweden). Nitrogen and protein components of human milk. Acta Paediatr Scand, 67: 561, 1978.—The true protein content of human milk is 0.9% in well nourished as well as malnourished mothers. Casein constitutes only about 20% of the protein nitrogen in human milk. The remaining 80% is derived from the whey proteins, the three dominant components being α-lactalbumin, lactoferrin and secretory IgA. α-lactalbumin is a subunit of lactose synthetase. Lactoferrin is an iron binding glycoprotein which plays a role in the defence against gastro-intestinal infections and is probably also involved in iron transport in the gut. Secretory IgA is comparatively stable at low pH; it is resistant to proteolytic enzymes and plays an essential role in the immunological defence against gastro-intestinal infections. Lysozyme is a minor component of the whey proteins and represents an active enzyme with a bactericidal effect. The nutritional and immunological significance of the marked differences with respect to the nitrogen and protein compositions of human milk and cow's milk should not be underestimated, but need further elucidation.

KEY WORDS: Human milk, protein components, infant nutrition

Our knowledge concerning the nitrogen and protein compositions of human milk and their variation has long been very limited. Many years ago Macy and her collaborators performed a series of outstanding studies on the composition of human milk (12, 13). At that time, however, the only available methods for qualitative and quantitative analysis of milk were those which were originally developed for the analysis of cow's milk. They were therefore not always applicable to human milk as there are physico-chemical differences between bovine and human casein which lead to different physico-chemical characteristics and subsequently different curd formation (15). This is of nutritional interest, since the curd formation influences the digestion of protein by the gastric enzymes in the stomach of the young infant.

On the other hand, certain findings by Macy and her group, e.g. the high content of non-protein nitrogen, have oddly enough been overlooked and not taken seriously into account. Thus it has been wrongly assumed that the protein content in human milk is 1.1 to 1.2% which represents an overestimation by 20 to 25% (8). The observation that 20–25% of the total nitrogen is in the form of non-protein nitrogen has since been verified in our studies on larger materials on well nourished as well as in malnourished mothers (9, 10).

Table I shows the nitrogen composition of human milk—both colostrum and mature milk. The data given in this table were obtained in a study on milk specimens from well nourished mothers, in which a complete analysis of the milk composition was performed (11). It is seen that non-protein nitrogen constitutes about 13% of the total nitrogen in colostrum but, as said before, about 25% in mature milk. The main part of the non-protein nitrogen is derived from urea, 11% and 33% respectively.

Table I. *Nitrogen composition of human milk*
The values refer to mg N per ml

	Colostrum	Mature milk
Total nitrogen	3.60	1.71±0.31
Protein nitrogen	3.13	1.29±0.[illegible]
Non-protein nitrogen	0.47	0.4 ±0.10
Urea nitrogen	0.05	0.14±0.03

Table 2 *Protein composition of human milk*
The values refer to mg protein per ml

	Colostrum	Mature milk
Casein	–	1 87±0 65
Lactoferrin	3 30	1 67±0 33
α lactalbumin	2 18	1 61±0 33
Secretory IgA	3 64	1 42±0 56
Lysozyme	0 34	0 38±0 04
Serum albumin	0 32	0 40±0 09

other nitrogen containing substances being creatine creatinine uric acid small peptides and free amino acids

The protein composition of milk specimens obtained from the same mothers is given in Table 2 For analytical reasons no value could be obtained for the casein content of colostrum It is quite obvious that α lactalbumin lactoferrin and secretory IgA constitute the dominating whey proteins in mature human milk Casein been stated earlier to constitute about 40% of the protein in human milk and the whey proteins the remaining 60% However the analyses performed at our laboratory by Lonnerdal (Table 2) show that the casein percentage in human milk might be as low as 20% This discrepancy might be due to the fact that a valid method for quantitative estimation of casein is still lacking At present casein is determined as the protein fraction which precipitates at pH 4 6 However if the nitrogen contents of the various human whey protein fractions i e α lactalbumin lactoferrin lysozyme serum albumin and the immunoglobulins (SIgA IgA IgM IgG) are substracted from the total nitrogen content there is only about 20% left to be made up by casein Our findings indicate that earlier values of 40% are due to the co precipitation of other protein fractions together with casein

α lactalbumin is a protein which is synthesized by the mammary gland It is one of the two proteins which form the lactose producing enzyme lactose synthetase (4) When milk specimens obtained from various mammals are compared it is obvious that the α lactalbumin content is related to the lactose content (4) As human milk is specially rich in lactose it also represents the milk that has the highest α lactalbumin content There is no direct correlation however between the lac

Table 3 *Nitrogen and protein contents of human milk and their variation during lactation*
The values refer to mg per ml

	Duration of lactation (months)				
	0–0 5	0 5–1 5	1 5–3 5	3 5–6 5	6 5–
Total nitrogen					
Swedish mothers	3 05	1 93	1 61	1 48	
Ethiopian priv	3 14	2 89	1 97		
Ethiopian non priv		2 50	1 77	1 69	1 74
Non protein nitrogen					
Swedish mothers	0 43	0 46	0 41	0 38	
Ethiopian priv	0 46	0 46	0 41		
Ethiopian non priv		0 43	0 36	0 34	0 33
Lactoferrin					
Swedish mothers	3 53	1 94	1 65	1 39	
Ethiopian priv	3 75	3 37	1 89		
Ethiopian non priv		2 64	1 67	1 72	1 48
α-lactalbumin					
Swedish mothers	3 62	3 76	2 78	2 68	
Ethiopian priv	3 70	3 72	2 92		
Ethiopian non priv		3 58	2 76	2 65	2 58

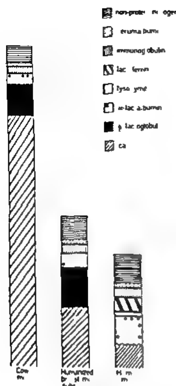


Fig 1 The protein composition of cow s milk humanized breast milk formula and human milk Nitrogen derived from different proteins and non protein nitrogen is given as gN/l (5)

tose and α lactalbumin contents and the α lactalbumin may not be the limiting factor for lactose as when α lactalbumin concentration which is under hormonal control decreases throughout lactation the lactose content increases

Lactoferrin is an iron binding glycoprotein which represents another major whey protein component in human milk It occurs in very low amounts in cow s milk It is also present in minor amounts in various other external secretions Lactoferrin specifically binds two ferric ions with incorporation of two molecules of bicarbonate It has been shown that lactoferrin is usually only saturated to a low degree with iron (14) Lactoferrin may play an essential role in the defence against gastro-intestinal infections by inhibiting the growth of Escherichia coli staphylococci and candida albicans (7) Since bacteria require iron for growth lactoferrin which mainly occurs in an unsaturated form in human milk probably binds iron so strongly that it makes it unavailable for the bacteria (1) It is also most probable that lactoferrin plays a role in the iron absorption in the infant intestines as it has been found that the iron in milk is absorbed to a much higher degree (50%) than iron in infant formula (2–10%) where it is in the form of ferrum reductum or easily absorbed iron salts (3 16)

Secretory IgA which constitutes the third major whey protein component is composed of two molecules of IgA which are covalently bound together to form a polypeptide the secretory component and the joining chain It is thus significantly different from the serum IgA The exact amount of IgA or secretory IgA is still difficult to evaluate owing to methodological problems Furthermore it has been difficult to define a standard as the SIgA/IgA ratio may vary As SIgA and IgA have the same type of antigenic determinants immunological methods have so far failed to be of use for the quantitative estimation of SIgA Lonnerdal at our laboratory has however developed a method based on affinity chromatography for the isolation of SIgA in human milk and according to this method its content in human mature milk should be about 1–2 mg/ml (11) Secretory IgA is very stable at low pH and comparatively resistant to proteolytic enzymes Furthermore intact SIgA has been shown to be present in the intestine of breast fed infants and it is suggested that it may participate in the defence against infection by binding viruses and bacteria and thereby preventing them from invading the mucosa (6)

Lysozyme represents another specific milk protein which occurs in a higher concentration in human milk than in milk from other species (2) It has a direct bactericidal effect by enhancing the activity of the immune antibodies Being an active enzyme the concentration is remarkably high

In addition to the milk specific proteins such

Table 2 *Protein composition of human milk*
The values refer to mg protein per ml

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The protein composition of milk specimens obtained from the same mothers is given in Table 2 For analytical reasons no value could be obtained for the casein content of colos trum *It is quite obvious that α lactalbumin* lactoferrin and secretory IgA constitute the dominating whey proteins in mature human milk Casein been stated earlier to constitute about 40% of the protein in human milk and the whey proteins the remaining 60% How ever, the analyses performed at our laboratory by Lonnerdal (*Table 2*) *show that the casein* percentage in human milk might be as low as 20% This discrepancy might be due to the fact that a valid method for quantitative esti mation of casein is still lacking At present *casein is determined as the protein fraction* which precipitates at pH 4 6 However if the nitrogen contents of the various human whey protein fractions i e α lactalbumin lacto ferrin lysozyme serum albumin and the im munoglobulins (SIgA IgA IgM IgG) are substracted from the total nitrogen content *there is only about 20% left to be made up by* casein Our findings indicate that earlier values of 40% are due to the co precipitation of other protein fractions together with casein

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Table 3 *Nitrogen and protein contents of human milk and their variation during lactation*
The values refer to mg per ml

	Duration of lactation (months)				
	0–0 5	0 5–1 5	1 5–3 5	3 5–6 5	6 5–
Total nitrogen					
Swedish mothers	3 05	1 93	1 61	1 48	
Ethiopian priv	3 14	2 89	1 97		
Ethiopian non priv		2 50	1 77	1 69	1 74
Non protein nitrogen					
Swedish mothers	0 43	0 46	0 41	0 38	
Ethiopian priv	0 46	0 46	0 41		
Ethiopian non priv		0 43	0 36	0 34	0 33
Lactoferrin					
Swedish mothers	3 53	1 94	1 65	1 39	
Ethiopian priv	3 75	3 37	1 89		
Ethiopian non priv		2 64	1 67	1 72	1 48
α-lactalbumin					
Swedish mothers	3 62	3 76	2 78	2 68	
Ethiopian priv	3 70	3 72	2 92		
Ethiopian non priv		3 58	2 76	2 65	2 58

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as casein α lactalbumin lactoferrin lysozyme and secretory IgA there are also proteins which are derived from the plasma such as the immunoglobulins IgG and IgM as mentioned earlier and serum albumin These proteins seem however to be of less practical importance from the nutritional and immunological points of view

Table 3 shows the variation in the contents of total nitrogen non protein nitrogen and the two milk specific proteins lactoferrin and α lactalbumin with time of lactations in Swedish well nourished mothers The contents of the specific proteins decrease during lactation in a way similar to the total nitrogen content while the non protein nitrogen is more constant The table also gives corresponding values obtained in milk specimens from well nourished Ethiopian mothers and it is obvious that these absolute values are about the same as are found in the Swedish mothers There is however, one most interesting exception that is regarding the lactoferrin content which is significantly higher in the specimens from Ethiopian mothers during the first 1 5 months This may be due to the fact that the diet of Ethiopian mothers is extremely rich in iron as a result of the high content of iron in the soil and in such staple cereals as tef It has been found that the daily iron intake corresponds to about 300 mg per day

Also given in the table are the corresponding values for milk specimens obtained from non privileged Ethiopian mothers In this case it was impossible for reasons of tribal traditions to get specimens before 2 weeks of lactation In view of the very poor intake of nutrients in the group of non privileged mothers which for almost all nutrients was below 60% of RDA it is remarkable that almost no qualitative differences with respect to the milk content of the investigated nutrients were observed However the mothers could not be described as severely malnourished

Lastly a comment upon the similarities and differences between the protein composition of cow s milk and human milk and its implication on the composition of human milk substitutes (Fig 1) The humanized substitutes or more accurately adapted formulas modified so far that they have a casein/whey protein ratio corresponding to 40/60 and a low content of minerals With regard to the protein versus non protein nitrogen content it is obvious that the absolute amount of non protein nitrogen is about the same in human milk as in cow s milk

In cow s milk β lactoglobulin is the dominating whey protein but this is completely lacking in human milk Whether or not this has an implication for the much discussed milk intolerance or allergy in infancy still has to be elucidated In human milk on the other hand lactoferrin is a major component while this is almost absent in cow s milk as also is lysozyme It is obvious that these discrepancies must also be reflected in the protein pattern of humanized breast milk substitutes (Fig 1) The significance of these differences needs to be further studied and evaluated

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Table 1 *Composition of milk lipids*

Duration of lactation	<1 month	2–6 months	7–12 months
Total lipids (g/100 g of milk)	3 ?±1 41	3 3±1 ?0	3 ?±1 30
Fatty acids (% of total)			
C	6 3±4 4	7 5±3 56	8 7±3 41
C	1? 8±5 31	11 1±4 21	14 2±6 15
C	6 8±8 56	?3 5±? 00	23 9±5 81
C	3 9±1 98	3 7±? 01	3 4±3 01
C	4 8±? 56	4 4±1 88	4 2±1 51
C	3? 9±7 03	35 3±8 11	27 9±6 19
C	11 1±6 3?	[illegible] 4±5 62	15 8±5 58

Mean ± S E

munised or because of illness of their older children Hence though the mothers were at tending the hospital they and the breast fed children were normal at the time of the milk collection Milk samples were collected from mothers at about 11 a m nearly 4 hours after the first feed and analysed for total fats The fatty acid composition of the milk fats was de termined The methyl esters of the fatty acids were separated by GLC at 180 C on poly Diethylene glycol succinate The distribution of fatty acids with 12 to 18 carbon units is shown in Table 1

Fatty acids with a chain length below 12 C could not be identified Fatty acids longer than 18 C were present in trace amounts The con centration of lipid and the fatty acid composi tion did not differ with duration of lactation except in colostrum In colostrum the fat con tent was higher and linoleic acid formed a lower percentage of the fatty acids When these values were compared with reported values from other countries it appeared that linoleic acid was slightly higher in our samples (Table 2)

The linoleate content of milk samples from Tanzanian women has been reported to be higher than in samples from European wom en (6)

The fat intake in Indian women is low but is almost entirely derived from vegetable sources Groundnut oil (peanut oil) is the most commonly consumed oil in these areas and In dian oil samples have been reported to contain about 25% linoleic acid (22) These analyses would indicate that a woman secreting 600 ml of milk per day (on an average) would be se creting about 3 g of linoleic acid which is equivalent to the amount present in 12 g of groundnut oil

Table 2 *The fatty acid composition of mature human milk lipids (% of total)*

Study	Saturated (1 –18 C)	Monoenes (16 1–18 1)	Dienes (18)	Ref No
Ind an	48 6	35 6	15 0	Present study
György	47 6	40 8	10 6	13
Breckenridge & Kuksis (1967)	4 6	41 6	10 0	5
Potter & Nestel (1976)				
S	44 6	45 9	8 7	25
P	39 1	45 5	14 7	
S	41 3	46 4	11 3	
[illegible]	37 3	4 7	19 2	
Bra co	41 4	39 3	6 5	3

Fatty acids above 20 C 3 0%
Periods when saturated fats were consumed
Periods when polyunsaturated fats were consumed

LIPID AND TRACE ELEMENT COMPOSITION OF HUMAN MILK

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ABSTRACT Belavady B (National Institute of Nutrition Indian Council of Medical Research Jamai Osmania Hyderabad India) Lipid and trace element composition of human milk Acta Paediatr Scand 67 566 1978 —The recent work on lipids and trace elements in human milk has been reviewed Lipids in human milk are specially important for the development of the central nervous system of the infant in addition to being a source of nutrients Recent studies revealed that the concentration of total lipids was low in milk samples from Indian women belonging to the low socio-economic group linoleic acid constituted a high percent of the fatty acids The bile stimulated lipase activity of milk was higher in Ethiopian than in Swedish milk samples Vitamin D sulphate the water soluble conjugate of vitamin D was present in higher concentrations than that reported for vitamin D in lipid fraction however the antirachitic activity of vitamin D sulphate is yet to be studied Copper zinc and magnesium concentrations were high in samples obtained during the first month of lactation Copper and zinc levels continued to fall gradually till the end of one year lactation The mean concentrations of zinc and copper in mature milk samples from Indian mothers were similar to those in American samples though both the samples showed wide variation A high proportion of zinc in milk was bound to proteins

KEY WORDS Human milk lipids lipase vitamin D trace elements

Human milk has been found to contain a large number of constituents The role of all these constituents is not yet defined though recent studies have identified the role of a number of them

Proteins and their fractions in milk have aroused a lot of interest and will form the subject of separate presentations at this symposium Of the other components in milk this paper will be confined to recent observations on lipids and trace elements

LIPIDS

Lipids in human milk derive their importance not only as sources of energy and as nutrients but also in relation to the development of the nervous system of the infant Experimental studies in animals have indicated that lipids play a crucial role during brain development (1 11) It has also been found that the young human infant is able to absorb fats from human milk more efficiently than those from cow s milk This difference may have two reasons—(1) the nature of the enzyme lipase and (2) the fatty acid composition of the triglycerides Since triglycerides form a very high percentage of the total lipid in milk the fatty acid composition of total lipids and triglycerides is similar It has been demonstrated that in human milk the beta position of triglycerides mainly contains palmitic acid while in cow s milk this position is occupied mostly by stearic acid (10) The human infant is able to absorb palmitic acid monoglyceride much more efficiently than stearic monoglyceride (9)

Studies on the composition of human milk fats are comparatively recent

Some studies have been carried out on the composition of lipids from milk of Indian women belonging to low socio economic groups (29) The mothers were apparently normal and were attending the local hospital for women and children to get their babies im

Table 5 *The trace element content of milk from Indian women (µg/ml)*

Duration	Sample size	Copper	Zinc	Magnesium
1 <1 month	96	0 48±0 011	4 76±0 60	36 58±1 ?48
1– 3 months	64	0 ?8±0 014	1 96±0 111	31 67±1 163
3 4– 6 months	80	0 _1±0 009	1 35±0 067	3? 59±1 200
4 7–1? months	59	0 17±0 009	1 04±0 069	30 43±1 356

Mean ± S E

a wide range of values for all trace elements investigated The iron content in milk has been studied most extensively and it is known that part of the iron is in the bound form in lactoferrin A recent study on iron absorption in infants from human milk or cow s milk formula indicated that the absorption from human milk was nearly five times as great as from cow s milk formula This was also confirmed by the observation that infants fed human milk had a better iron nutritional status (28) Zinc is also known to be present bound with protein both in human milk and in fresh cow s milk (20) Zinc was precipitated with the casein fraction and the globulin fraction The proteins in human and bovine milk have been recently separated on columns and it has been observed that in human milk a high proportion of zinc is associated with low molecular weight protein fractions while in cow s milk it is associated with *high molecular* weight protein (8) The different proteins with which zinc is bound in the two milks may have physiological significance The zinc status of babies suffering from the genetic disorder Acrodermatitis Enteropathica which usually occurs when infants are weaned from human to cow s milk is poor Human milk had a therapeutic value in this condition (4) Incidentally it was observed that the zinc concentration in milk powder was lower and the form in which it existed in the powder was different from that in fresh cow s milk While zinc was present predominantly in the bound form in fresh milk it was present mainly in the free form in milk powder The form in which the other trace elements are present in human milk is not known The significance of the form in which it is present in relation to the nutrition of the infant is a subject which has not received much attention Trace element nutrition has evoked a great deal of interest in recent years and it is hoped that human milk will receive its due consideration in future studies The interrelation between the nutritional status of the mother and the content of trace elements in milk is one such area Most of the reports available pertain to the total trace element content of the milk with no reference to the form in which they are present Recently studies have been carried out on the Cu Zn and Mg contents of human milk (21 26) The trace element composition of human milk at different stages of lactation is shown in Table 5 (29)

It was noted that both the copper and zinc concentrations showed a significant reduction in samples collected after one month The decline continued till about 12 months but was gradual The changes in magnesium were minimal compared to copper and zinc The copper and zinc concentrations in serum were also studied in these women The copper concentration which was around 2 1 µg/ml soon after parturition had decreased to about 1 2

Table 6 *Trace element content of human milk samples from Americans (µg/ml)*

Duration to lactation	Copper	Iron	Zinc
6–1 weeks			
Mean	0 4	0 71	1 6
Range	0 09–0 63	0 1–1 6	0 14–3 95

Table 3 *Bile-salt stimulated (B Ss) and serum stimulated (S S) lipases in human milk samples (µkat/litre)*

E N P = Ethiopian non privileged E P = Ethiopian privileged

Duration of lactation	Lipases	E N P	E P	Swedish
0 3–3 5 months	B Ss	640	871	462
	S S	3 0	1 9	1 7

LIPASES

Human milk has been reported to contain two lipases—one stimulated by bile salt and the other by serum both the lipases have been characterised (14 15) Their activities have been quantitated in recent years in milk samples from women of low and high socio economic groups (16) (Table 3)

The results indicated that the bile salt stimulated lipase activity was very much higher than the serum stimulated activity The milk samples from Ethiopian privileged mothers showed higher mean lipase activity than the poor Ethiopian women and the activity in both these groups was higher than in the Swedish mothers however these differences were not significant due to the wide variations in all groups The bile salt stimulated lipase activity did not differ significantly between the different groups The implication of these differences is yet to be understood in relation to the nutritional status and the dietary pattern of the mothers and the nutrition of the infant

Assays for vitamin D activity in human milk have conventionally been confined to the lipid fraction and this has been reported to be low in milk (7 19 24) However the identification of vitamin D sulphate in the fat free protein free filtrate of human milk has created new interest in the vitamin D activity of milk (18 27)

Lakdawala & Widdowson (17) have studied the vitamin D activity in milk samples and demonstrated considerable amounts of vitamin D sulphate in the aqueous phase The concentrations of vitamin D sulphate at different stages of lactation as reported by these workers are shown in Table 4

The concentration in colostrum was high but decreased with the duration of lactation The values obtained in this study are higher than those found in the lipid fraction by earlier workers However the biological activity of vitamin D sulphate and its availability to the infants remain to be investigated The development in this field will be watched with interest

Earlier studies had indicated low levels of vitamin A in milk samples of under nourished women as compared with well nourished groups Colostrum was a rich source of vitamin A (2) Vitamin A in milk and retinol binding protein in serum were studied in groups of well nourished Swedish mothers and privileged and non privileged Ethiopian mothers Vitamin A levels were lower in milk samples from the under privileged Ethiopian women than from groups of privileged mothers the retinol binding protein in plasma was also low in non privileged Ethiopian women (12)

TRACE ELEMENTS

The data available concerning trace element concentrations in human milk samples reveal

Table 4 *Vitamin D sulphate in human milk (µg/100 ml)*

Duration of lactation	Vitamin D sulphate
3– 5 days	1 8±0 39
6– 8 days	1 0±0 29
29–42 days	0 9±0 40

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CONTAMINANTS IN HUMAN MILK

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ABSTRACT Olszyna Marzys A E (Institute of Nutrition of Central America and Panama Guatemala City Guatemala C A) Contaminants in human milk Acta Paediatr Scand 67 571 1978.—There is a paucity of information regarding excretion of contaminants in *human milk due to* experimental difficulties and until recently a general lack of interest Because of the high fat content of milk and as its acidity is higher than *that of plasma nearly* all liposoluble and basic agents consumed by the mother will be *excreted in the milk* Distinction must be made between on the one hand drugs and social toxicants such as smoking and alcohol whose intake can be stopped or limited during pregnancy and lactation and ecological toxicants *present in* a polluted en ironment to which the mother is exposed Cases have occurred *of heavy prenatal* and postnatal intoxication of infants with hexachlorobenzene in Turkey and methylmercury in Iraq due to consumption of fungicide treated seed wheat by *pregnant and lactating* mothers Recent attention has been concentrated on contamination of milk with *organochlorine* compounds such as DDT and PCB s that are found in many parts of the world The heaviest *contamination* with DDT has been found in Guatemala resulting in suckling infants consuming *many times the Acceptable* Daily Intake of this compound pro posed by WHO with unknown future effects

KEY WORDS Human milk contaminants *toxicants pesticides*

Owing to experimental difficulties and until recently a general lack of interest there is a paucity of information regarding excretion of contaminants in human milk

As Knowles entitled his second recently published review of the problem of excretion of drugs in milk Breast milk is a source of more than nutrition for the neonate (9) Near ly all agents ingested by the mother will be found in her milk in some form or other Their distribution across the membrane between plasma and milk is influenced by their con centration solubility in fats and water pKa or degree of ionization and transport mech anisms

Since human milk is relatively high in fat liposoluble drugs will tend to concentrate in it Because its acidity is higher than that of the plasma concentration of basic components in the milk will be promoted

μg/ml after about one month of lactation The serum concentration of zinc remained constant The correlation between the serum and milk concentrations of copper and of zinc was not high On the other hand the copper and zinc concentrations in milk showed some correlation ($r=0.61$ and $p<0.001$) The copper zinc and magnesium concentrations were within the ranges reported for human milk Factors influencing the trace element composition are not known From a recent study on copper iron and zinc levels in milk the day to day diurnal and weekly variations in the composition have been reported (23) Copper and iron showed wide variations while the concentration of zinc was more consistent The ranges observed in this study are given in Table 6

An earlier report on the content of some of the trace elements in mature milk in another state in India had revealed significantly higher values for zinc (2) Large areas of cultivated land in Andhra Pradesh are reported to be zinc deficient the influence of this deficiency on the zinc content in grain is yet to be studied Extensive investigations are required in this field to understand the relationship between the trace element composition of milk and the dietary intake of these nutrients in the mothers on the one hand and the health of the infants on the other

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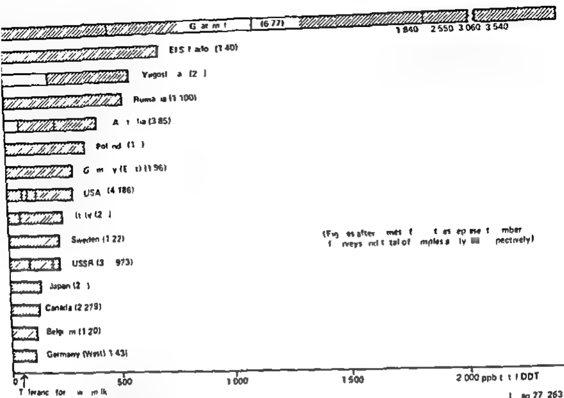


Fig ? Averages of total DDT content of human milk found in various countries 1961–1975 (in parts per billion (1000 millions))

human mothers excrete some 12 5% of the in take of this compound while cows excrete only about 1 5% Human milk has always been found to contain more DDT than cow s milk (9)

Other factors to be considered when pre scribing medication are how and to what ex tent the agent in question (or its metabolites) may affect the child and the possibility that its known presence may still be more innocuous than that of unknown products which might exist in substitutes for mother s milk

In any case with medication we are more or less in control of the situation The problem may be more difficult with social toxicants Al cohol and barbiturates taken separately in moderate amounts are said not to affect the suckling child Cigarette smoking results in passing alone some 0 4–0 5 mg of nicotine one of the most toxic drugs in each litre of milk in the case of mothers smoking ten to twenty cigarettes a day with a variety of physiological effects And yet apart from the known diffi culty of stopping smoking there is the added difficulty in convincing heavily smoking moth ers that doing so during pregnancy and lacta tion may affect the infant

If cigarette smoking by the lactating mother may be bad for the child marijuana smoking may result in a real tragedy and more and more documented cases showing what this potent drug can do to a baby are coming to light

However it is over ecological toxicants that neither the mother nor other individuals as such have much control In some cases of massive intoxication of the mother followed by prenatal and postnatal transmission of the toxicants to the foetus and then to the suckling infant the causes of the tragedies have been a combination of poverty ignorance lack of adequate precautions and use of highly toxic

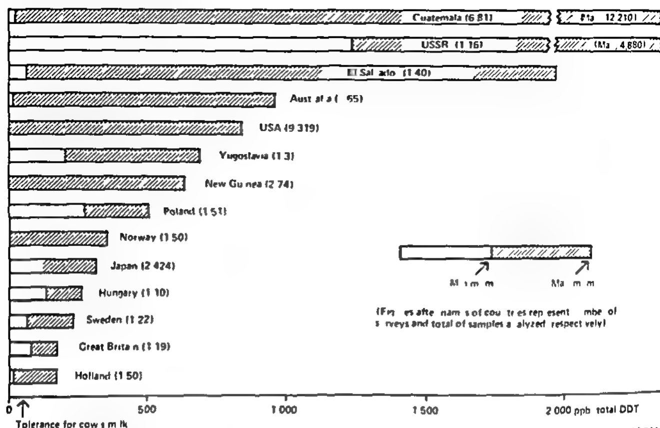


Fig 1 Maxima and minima of total DDT content of human milk found in various countries 1964–1976 (in parts per billion (1 000 millions))

Knowles (8) has tabulated literature references to nearly sixty compounds or classes of compound whose excretion in the milk has been reported in human or animal studies Arena (2) also talks about oral contraceptives and social toxicants such as alcohol and smoking and includes pesticides among ecological toxicants

It is necessary to emphasize the difference between this last group of contaminants on the one hand and drugs and social toxicants on the other When required the ingestion of drugs and smoking or drinking can be stopped or limited voluntarily or by persuasion by the individual Ingestion of ecological toxicants by exposure to environmental contamination can only be reduced by concerted action of the society and authorities

However even in the case of drugs and social toxicants while the perils of medication smoking drinking and narcotic drugs during gestation are being increasingly recognized and caution exercised in the prescription of drugs during pregnancy especially since the thalidomide tragedy there is much less hesitancy in prescribing them during the period of lactation This is perhaps due to the fact that as we know in most industrialized countries breast feeding has been drastically reduced or eliminated so that not much attention is paid to that period by most members of the medical community However in developing countries breast feeding is still predominant and may be continued for eighteen or even more months after birth

When medication is indicated for the mother during the lactation period several factors have to be taken into consideration In some cases such as that of radioactive iodine the amount of drug excreted is considerable whereas in others only minute quantities pass into the milk (3) In this respect there may be a considerable difference between different species In the case of DDT for instance

eptachlor epoxide	
ean ± S D (ange)	Positive samples
0 007±0 007 (0-0 008)	19
d	
0 003±0 007 (0-0 01)	3

mother to foetus resulting in higher blood mercury levels in the foetus than in its mother and that neonatal blood mercury levels are maintained through ingestion of mercury in the mother s milk resulting in clinical manifestations of methylmercury poisoning

Although no similar cases of intoxication have been described in the literature in the case of organochlorine compounds it is the contamination with these that has been the subject of most studies of contamination of human milk since the first one in 1951 (10) Figs 1 and 2 summarize the levels of DDT its metabolites and isomers found in human milk in various countries Apart from insecticides several more recent studies also include non pesticidal polychlorinated biphenyls (PCB) and similar industrial contaminants In addition to the results shown in the tables series of studies have been carried out since

ptachlor epoxide	
an S D (ange)	Positive samples
001 0 00 (001-0 004)	[illegible]

1970 in all the prefectures of Japan showing the main contaminant to be the isomers of BHC (HCH) and not DDT (7)

As far as DDT is concerned the highest values published to date have been those in Guatemala reported by the present author and his collaborators The results of the first study carried out in 1970–1971 and published in 1973 (11 12) are summarized in Table 1

Extremely high contamination was found in three rural localities two of them in cotton growing areas Based on the practical residue limit of 0 05 ppm of DDT in commercial milk and the WHO recommended acceptable daily intake (ADI) of 0 005 mg per kg body weight for adults the figures meant that human milk in Guatemala contained between 7 and 244 times the maximum DDT concentration legally tolerated in the USA and a number of other countries and that taking into account the average amount of mother s milk consumed daily by a Guatemalan child between 6 and 207 times the amount of DDT considered by WHO as acceptable would be ingested by these infants

Two further studies one in three other localities in Guatemala and one in neighbouring El Salvador showed levels of the same order (Tables 2 and 3) (4) The levels were higher in rural than in urban areas and higher in cotton growing areas than in areas without cotton However it is considered that massive indiscriminate and ever increasing (tenfold per acre in as many years) spraying of cotton fields most of it aerial has been mainly responsible for the heavy contamination of the Central American Isthmus with DDT (6)

Our findings have since helped to promulgate legislation on the use of pesticides in Guatemala with DDT scheduled to be phased out over three years If it is suitably enforced the DDT levels in the population should gradually fall However in the meantime the possible effect of this massive feeding of babies with DDT during the first months of their life is not known Claims of innocuity of exposure to DDT as made by formulators and sprayers

Table 1 *Chlorinated pesticides in human milk—Guatemala C.A.*

Values expressed in ppm whole milk basis. S.D. = standard deviation. n.d. = not detected. Values below 0.001 ppm are not reported

Community	Crop	No. of samples	Total DDT Mean ± S.D. (Range)	Total DDT Positive samples	Total HCH Mean ± S.D. (Range)	Total HCH Positive samples	Dieldrin Mean ± S.D. (Range)	Dieldrin Positive samples
El Rosario Champerico	*Cotton, corn, sesame*	27	1.84±1.25 (0.342–4.97)	27	0.006±0.005 (0–0.019)	23	0.002±0.001 (0–0.010)	23
Cerro Colorado La Gomera	Corn, cotton	9	3.06±1.81 (1.57–6.68)	9	0.015±0.019 (0–0.057)	5	n.d.	
La Bomba Chiquimulilla	Corn	10	1.11±0.80[b] (0.411–1.77)	10	0.0024±0.009[d] (0.010–0.035)	10	n.d.	

[a] Excluding one sample containing 12.21 ppm
[b] Excluding one sample containing 11.50 ppm
[c] Excluding one sample containing 0.069 ppm
[d] Excluding one sample containing 0.101 ppm

Table 2 *Chlorinated pesticides in human milk—Guatemala C.A.*

Values expressed in ppm whole milk basis. S.D. = standard deviation. n.d. = not detected. Values below 0.001 ppm are not reported

Community	Crop	No. of samples	Total DDT Mean ± S.D. (Range)	Total DDT Positive samples	Dieldrin Mean ± S.D. (Range)	Dieldrin Positive samples	Heptachlor epoxide Mean ± S.D. (Range)	Heptachlor epoxide Positive samples
Guatemala City	–	15	0.490±0.345 (0.025–1.03)	15	n.d.		n.d.	
Morales Izabal	Banana	10	2.55±1.68 (1.14–6.60)	10	0.005	1	0.002	1
Escuintla	Cotton	10	3.54±2.55 (0.600–9.26)	10	0.070	1	n.d.	

compounds that could have been replaced by more harmless ones. Such were the cases for instances in children of nursing mothers who had eaten hexachlorobenzene treated seed wheat in Turkey in 1956, and those in Iraq in 1972 whose mothers had consumed home made bread prepared from wheat treated with a methylmercury fungicide—in this latter case a repetition of the Guatemalan intoxication in 1956 (13). Studies conducted by Amin Zaki et al. (15) during the first seven months after the poisoning on 15 infant mother pairs exposed to methylmercury during pregnancy showed that this substance passes readily from

Table 3 *Chlorinated pesticides in human milk—El Salvador C.A.*

Values expressed in ppm whole milk basis. S.D. = standard deviation

Community	Crop	No. of samples	Total DDT Mean ± S.D. (Range)	Total DDT Positive samples	Total HCH Mean ± S.D. (Range)	Total HCH Positive samples	Dieldrin Mean ± S.D. (Range)	Dieldrin Positive samples
Santiago de Maria El Salvador	Coffee	40	0.695±0.460 (0.062–1.96)	40	0.012±0.010[b] (0.001–0.040)	27	0.005±0.004 (0.001–0.015)	23

[a] Excluding one sample containing 5.50 ppm
[b] Excluding one sample containing 0.089 ppm

eptachlor epoxide	
ean ± S D (ange)	Positive samples
007±0 00? -0 008)	19
d	
0 003±0 007 0-0 0 1)	3

mother to foetus resulting in higher blood mercury levels in the foetus than in its mother and that neonatal blood mercury levels are maintained through ingestion of mercury in the mother s milk resulting in clinical manifestations of methylmercury poisoning

Although no similar cases of intoxication have been described in the literature in the case of organochlorine compounds it is the contamination with these that has been the subject of most studies of contamination of human milk since the first one in 1951 (10) Figs 1 and 2 summarize the levels of DDT its metabolites and isomers found in human milk in various countries Apart from insecticides several more recent studies also include non pesticidal polychlorinated biphenyls (PCB) and similar industrial contaminants In addition to the results shown in the tables series of studies have been carried out since

leptachlor epoxide	
lean ± S ■ Range)	Positive samples
0 003 _0 00? 0 001-0 004)	

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Two further studies one in three other localities in Guatemala and one in neighbouring El Salvador showed levels of the same order (Tables 2 and 3) (4) The levels were higher in rural than in urban areas and higher in cotton growing areas than in areas without cotton However it is considered that massive indiscriminate and ever increasing (tenfold per acre in as many years) spraying of cotton fields most of it aerial has been mainly responsible for the heavy contamination of the Central American Isthmus with DDT (6)

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El Rosario Champerico	Cotton corn sesame	27	1 84±1 25 (0 342–4 97)	27	0 006±0 005 (0–0 019)	23	0 002±0 003 (0–0 010)	23
Cerro Colorado La Gomera	Corn cotton	9	3 06±1 81 (1 57–6 69)	9	0 015±0 019 (0–0 057)	5	n d	
La Bomba Chiquimulilla	Corn	10	1 11±0 80[b] (0 411–1 77)	[illegible]	0 0034±0 009[d] (0 010–0 035)	10	n d	

Excluding one sample containing 12 21 ppm
[b] Excluding one sample containing 11 50 ppm
Excluding one sample containing 0 069 ppm
[d] Excluding one sample containing 0 101 ppm

Table 2 *Chlorinated pesticides in human milk—Guatemala C A*

Values expressed in ppm whole milk basis S D =standard deviation n d =not detected Values below 0 001 ppm are not reported

Community	Crop	No of samples	Total DDT Mean ± S D (Range)	Total DDT Positive samples	Dieldrin Mean ± S D (Range)	Dieldrin Positive samples	Heptachlor epoxide Mean ± S D (Range)	Heptachlor epoxide Positive samples
Guatemala City	–	15	0 480±0 345 (0 025–1 03)	15	n d		n d	
Morales Izabal	Banana	10	2 55±1 68 (1 14–6 60)	10	0 005	1	0 002	1
Escuintla	Cotton	10	3 54±2 55 (0 600–9 26)	10	0 070	1	n d	

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Values expressed in ppm whole milk basis S D =standard deviation

Community	Crop	No of samples	Total DDT Mean ± S D (Range)	Total DDT Positive samples	Total HCH Mean ± S D (Range)	Total HCH Positive samples	Dieldrin Mean ± S D (Range)	Dieldrin Positive samples
Santiago de Maria El Salvador	Coffee	40	0 695±0 460 (0 062–1 96)	40	0 012±0 010[a] (0 001–0 040)	27	0 005±0 004 (0 001–0 015)	23

Excluding one sample containing 5 50 ppm
[a] Excluding one sample containing 0 089 ppm

ptachlor epoxide	
ean ± S D (ange)	Positive samples
007±0 00 -0 008)	19
d	
003±0 007 -0 0 1)	3

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Heptachlor epoxide	
Mean±S D (Range)	Positive samples
0 003 _0 00 0 001–0 004)	

1970 in all the prefectures of Japan showing the main contaminant to be the isomers of BHC (HCH) and not DDT (7)

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It is only to be hoped that the increasing consciousness of the problem will lead to the adoption of measures with which to tackle it

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NEW KNOWLEDGE IN HUMAN MILK IMMUNOGLOBULIN

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ABSTRACT Hanson L Å Ahlstedt S Carlsson B Fallstrom S P Kaijser B Lindblad B S Sohl Åkerlund A and Svanborg Eden C (Department of Immunology Institute of Medical Microbiology University of Goteborg Goteborg Sweden Department of Paediatrics University of Goteborg Goteborg Sweden and Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) New knowledge in human milk immunology Acta Paediatr Scand 67 577 1978.—One of the anti infection principles of maternal milk is the predominant milk immunoglobulin secretory IgA This immunoglobulin contains antibodies against many pathogens and potential pathogens viruses as well as bacteria including several members of *Enterobacteriacae* The antigenic stimuli for these milk antibodies seem to take place in the Peyer s patches of the intestine Lymphoid cells leaving the patches after antigenic exposure seem to home to the mammary glands via the lymph and blood circulation As a result the milk contains secretory IgA antibodies against among other things the intestinal bacteria of the mother These milk antibodies might reflect the spectrum of bacteria and viruses in the community and may be important for the protection of the breast fed baby Via the same homing mechanism the maternal milk obtains antibodies against dietary antigens including cow s milk proteins Studies of infants on mixed feeding suggest that the secretory IgA antibodies against the bovine proteins diminish the antigenic exposure indicating the possibility of an anti allergic mechanism

KEY WORDS Breast feeding local immunity secretory IgA antibodies vaccination

Findings in several studies seem to support the notion that breast feeding can protect the infant against infections (12 14 15 25) Since many factors may influence the occurrence of infections such as the hygienic conditions and the nutritional situation it has been difficult to define in detail the role of various factors in the human milk in such protection The observations by Mata and Urrutia from Guatemala are striking however indicating that although the breast fed babies are exposed to pathogens such as *Shigella Salmonella* and enteropathogenic *E coli* they may have no symptoms of infections—as long as they are breast fed (21) The high frequency of infections appearing after weaning may be a result of the simultaneous disappearance of the maternal protection the increased intake of microorganisms with other foods and malnutrition

Human milk contains many factors which may add to its antiinfectious properties In addition to the iron binding lactoferrin lysozyme bifidus factor phagocytes and T and B lymphocytes specific antibodies are also present (1 2 9 10 12 14 15 25 27 31) The latter are dominated by the secretory IgA antibodies which are part of a system for protection of the mucous membranes of the body New information has recently been provided concerning the production and mode of function of the secretory IgA antibodies and this is reviewed below

The secretory IgA of human milk

Quantitation of milk secretory IgA which is usually present together with 7S IgA and secretory component as well as with IgA aggregates is a difficult technical problem (15) This was recently overcome by the development of

apply to adults not infants A high prevalence of malnutrition infectious diseases and other negative factors in the area would make the isolation of any effects and specific attribution of them to DDT rather problematic Nevertheless, the synergistic effect of these factors on insults presented by other potential toxicants is well known and it is difficult to imagine that the addition of such massive contamination of their only food to their other miseries would have no ill effect whatever on the children

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NEW KNOWLEDGE IN HUMAN MILK IMMUNOGLOBULIN

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ABSTRACT Hanson L Å Ahlstedt S Carlsson B Fallstrom S P Kaijser B Lindblad B S Sohl Åkerlund A and Svanborg Eden C (Department of Immunology Institute of Medical Microbiology University of Goteborg Goteborg Sweden Department of Paediatrics University of Goteborg Goteborg Sweden and Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) New knowledge in human milk immunology Acta Paediatr Scand 67 577 1978.—One of the anti infection principles of maternal milk is the predominant milk immunoglobulin secretory IgA This immunoglobulin contains antibodies against many pathogens and potential pathogens viruses as well as bacteria including several members of *Enterobacteriacae* The antigenic stimuli for these milk antibodies seem to take place in the Peyer s patches of the intestine Lymphoid cells leaving the patches after antigenic exposure seem to home to the mammary glands via the lymph and blood circulation As a result the milk contains secretory IgA antibodies against among other things the intestinal bacteria of the mother These milk antibodies might reflect the spectrum of bacteria and viruses in the community and may be important for the protection of the breast fed baby Via the same homing mechanism the maternal milk obtains antibodies against dietary antigens including cow s milk proteins Studies of infants on mixed feeding suggest that the secretory IgA antibodies against the bovine proteins diminish the antigenic exposure indicating the possibility of an anti allergic mechanism

KEY WORDS Breast feeding local immunity secretory IgA antibodies vaccination

Findings in several studies seem to support the notion that breast feeding can protect the infant against infections (12 14 15 25) Since many factors may influence the occurrence of infections such as the hygienic conditions and the nutritional situation it has been difficult to define in detail the role of various factors in the human milk in such protection The observations by Mata and Urrutia from Guatemala are striking however indicating that although the breast fed babies are exposed to pathogens such as *Shigella Salmonella* and enteropathogenic *E coli* they may have no symptoms of *infections*—as long as they are breast fed (21) The high frequency of infections appearing after weaning may be a result of the simultaneous disappearance of the maternal protection the increased intake of microorganisms with other foods and malnutrition

Human milk contains many factors which may add to its antiinfectious properties In addition to the iron binding lactoferrin lysozyme bifidus factor phagocytes and T and B lymphocytes specific antibodies are also present (1 2 8 10 12 14 15 25 27 31) The latter are dominated by the secretory IgA antibodies which are part of a system for protection of the mucous membranes of the body New information has recently been provided concerning the production and mode of function of the secretory IgA antibodies and this is reviewed below

The secretory IgA of human milk

Quantitation of milk secretory IgA which is usually present together with 7S IgA and secretory component as well as with IgA aggregates is a difficult technical problem (15) This was recently overcome by the development of

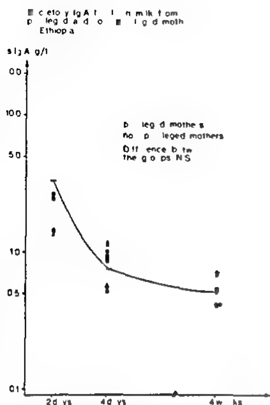


Fig. 1. Diagram showing the similar levels of secretory IgA in milk from privileged and non privileged mothers from Ethiopia. A modified enzyme linked immunosorbent assay was employed, eliminating the technical problems often introduced by other methods such as radial immunodiffusion for measurements of IgA in secretions (7).

a modification of the enzyme linked immunosorbent assay (32). Employing this technique we found no differences in the secretory IgA levels between maternal milk from privileged and non privileged mothers in Ethiopia (Fig. 1).

Furthermore, the levels corresponded to those found in milk from healthy Swedish mothers (7). These findings are in accord with our earlier results of determinations of milk IgM, IgG and secretory IgA antibodies to *E. coli* in milk from undernourished Pakistani and healthy Swedish mothers (4). There were no differences in antibodies against common *E. coli* O antigens, but due to the epidemiological situation the levels of milk antibodies against O antigens of enteropathogenic *E. coli* were much higher in the Pakistani samples (2). These findings suggest that the concentration of antibodies in milk may not be diminished in the undernourished mothers. There was some suggestion, however, that their milk volume might have been diminished, which could result in an immunological as well as a nutritional deficiency in their breast fed offspring (4).

The rapid fall of the IgA level in the milk after parturition has given the impression that insignificant amounts of antibodies are provided to the breast fed baby. However, the simultaneous increase in volume seems to compensate for the decrease in concentration of the immunoglobulin. Throughout lactation there seems to be a relatively constant and significant output of secretory IgA antibodies (5, 10, 13, 25, 30).

Studies of the antibodies in human milk have shown secretory IgA antibodies against viruses, including polio virus (17, 29), and a series of microbial antigens, such as *E. coli* O and K antigens (2, 4, 5, 10, 23), *Shigella* O antigen (6) and *V. cholerae* O antigen (35), and enterotoxins from *E. coli* and *V. cholerae* (18). Investigations of the IgA producing B lymphocytes found in human milk have also illustrated that a large proportion of the milk cells produce antibodies against enterobacterial antigens (1).

It is not clear how milk can contain antibodies against antigens present in the intestine which hardly come into contact with the lymphoid tissue of the mammary gland, where the local synthesis of the secretory IgA milk antibodies takes place. Recent studies have brought some light to this question.

The production of the milk secretory IgA antibodies

Secretory IgA antibodies are composed of dimers of IgA with an additional polypeptide, the J chain. These molecules are produced by B lymphocytes which are found adjacent to epithelial cells of exocrine glands, such as the submucosal glands in the intestine and the respiratory tract. They are also found in the mammary and the salivary glands, as well as in the lacrimal glands. The J IgA dimers leaving the lymphocytes seem to pass through the

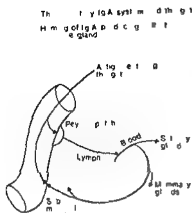


Fig 2 Schematic diagram showing the presumed homing mechanism of the secretory IgA antibody sy tem for local protection of mucous membranes The Peyer s patches function as a central organ where the IgA producing cells originate After stimulation by antigen from the intestine cells leave via the lymph reach the blood and home to exocrine glands including the mammary glands where secretory IgA antibodies are produced

epithelial cells of the glands and to be provided there with a glucopeptide the secretory com ponent (SC) The resulting composite mole cule is more resistant to enzymic degradation and pH variations than serum antibodies (15)

The milk IgA seems to be produced in the mammary gland by cells which are part of the generalized system of special B lymphocytes These cells produce the IgA dimers which combine with J and SC chains to result in the complete secretory IgA molecules The Peyer s patches in the small intestine play a central role in this system (8 15 28) The epithelium of the Peyer s patches seems to present antigens from the gut to lymphoid cells of the patches Antigen stimulated lymphoid cells then leave this organ and travel via the lymph the mesenteric lymph glands and the thoracic duct into the blood By a yet unknown homing mechanism the cells seem to relay to the tissues of exocrine glands including the mammary gland (Fig 2)

This concept has been produced on the basis of studies showing that lymphocytes from the Peyer s patches can repopulate the intestinal mucosa of irradiated animals (8) Furthermore the intestinal immunization of rabbits was shown to result in the appearance of antibodies in the milk (26) *Salmonella typhimurium* infection during lactation gave rise to antibodies in the milk of humans (3) The colonization of the intestinal tract of pregnant women resulted a few days later in production by cells appearing in the milk of secretory IgA antibodies against the O antigen of the employed *E coli* strain (11) Likewise vaccination of pregnant women with a live *Shigella* vaccine was followed a few days later by appearance in the milk of cells producing secretory IgA antibody against the O antigen of the vaccine strain (6) Since there was no simultaneous serum antibody response (6 11) it is most likely that the antigen was not trans ported to the mammary gland via the blood but that lymphoid cells homing to the mam mary gland after the antigenic exposure in the gut were responsible This view was strongly supported by recent experimental studies by Roux et al (28) of the homing mechanism of IgA producing cells in mice This group also showed that the homing was dependent on the hormonal situation (38) On administration of the hormones prolactin oestrogen and pro- gesteron to the mice they showed that the Peyer s patch lymphocytes very efficiently located to the mammary gland It was hypo- thesized that the hormones induced or in creased the number of some kind of tissue re ceptors for the IgA producing cells

As a consequence of the homing of the IgA producing lymphocytes from the intestine to the mammary gland the milk antibody content also reflects the antigenic exposure and local antibody response in the gut The breast fed baby is therefore provided with milk IgA anti bodies against the microorganisms harboured by or passing through the mother s intestine and also against those present in the environ ment and likely to reach the baby The milk antibody content thus reflects the local epi demiological situation as it provides a list of the microbial agents present in the area where the mother lives Finally the milk antibodies

Table 1. *Inhibition of E. coli 06 adherence to uroepithelial cells by human milk and γ globulin containing corresponding O antibodies*

Addition to bacteria and epithelial cells	Mean no. of bacteria per epithelial cell	Level of significance	Antibody concentration ELISA	
			IgG	SIgA
PBS	59		–	–
		$p<0.01$		
Human milk	3		<1	3 [illegible]
		$p<0.01$		
Human milk absorbed with 06 LPS	25			1.1

can be used to follow the intestinal response to vaccines.

Stimulation of milk antibodies by vaccination

The possibility of boosting the milk antibody level by vaccination was recently illustrated by studies on lactating Pakistani mothers (35). These mothers already had a local antibody response in the form of SIgA antibodies in milk and saliva, presumably due to natural exposure to *V. cholerae*. Subcutaneous vaccination with a cholera vaccine resulted not only in the expected serum antibody response, but also in an increase in SIgA antibodies in milk and saliva. These findings demonstrate that protection can be induced simultaneously in the mother's gut and in her breast fed baby since protection against cholera is most probably provided primarily by local immunity (19). Obviously it may be possible to direct this local immunity, including the milk antibodies. This is of great interest in the face of the recent development of a very promising new cholera vaccine (19, 20).

A further development of vaccines depending on their effect on local immunity can be foreseen. This is based on the fact that the function of the locally produced antibodies is to prevent the adhesion of bacteria to mucous surfaces that is often necessary for infection to take place. An anti adherence effect on *E. coli* has recently been found in human milk (Table 1) (34). These adherence preventing antibodies may be directed against O antigens as well as special adherence structures, pili, on the bacteria. It may be possible to develop efficient, topically applied vaccines composed of pili antigens in combination with other surface structures such as O and K antigens.

Do milk secretory IgA antibodies protect against allergies?

According to Soothill (33), the secretory IgA antibodies may provide an antigen avoidance system. It has been suggested that one of the reasons for an increased risk of the development of allergies in infants with atopic parents may be a low IgA level early in life (36). In fact, Walker and Isselbacher showed that locally produced intestinal antibodies diminished the uptake of native proteins in the gut of experimental animals (37). It is as yet unknown when the normal infant can produce sufficient local antibodies in the gut against potential allergens to diminish the risk of sensitization. It seems possible that the maternal milk could provide such allergy protecting antibodies in early life. Human milk contains antibodies against cow's milk proteins (2, 16, 24). Obviously the IgA producing cells from the Peyer's patches are induced not only by microbial but also by dietary antigens.

A comparison was made between infants transferred directly from maternal milk to cow's milk based formulas and those with a period of mixed feeding with breast feeding as well as formulas. It was found that the latter infants had a significantly smaller serum antibody response to cow's milk proteins, sug

gesting that the maternal milk SIgA antibodies may have diminished the exposure to the potential allergens of the formulas (2 16)

Further studies are required to define a pre sumptive antiallergic role of breast feeding Recent results of Soothill s group strongly sug gest that elimination of potential allergens from infant food lowers the frequency of aller gic reactions (22) Breast feeding both largely eliminates possible allergens and provides an tibodies that may help to protect against the al lergens of mixed feeding

ACKNOWLEDGEMENTS

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THE VOLUME OF TRAPPED GAS A NEW AND SENSITIVE TEST FOR THE DETECTION OF EXERCISE INDUCED BRONCHOSPASM IN CHILDREN

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ABSTRACT Svenonius E Lecerof H Lilja B and Arborelius M Jr with technical assistance of Mrs Rosie Kautto (Departments of Paediatrics and Clinical Physiology University of Lund Malmo General Hospital Malmo Sweden) The volume of trapped gas A new and sensitive test for the detection of exercise-induced bronchospasm in children Acta Paediatr Scand 67 583 1978.—The volume of trapped gas (VTG) was measured at the end of a nitrogen multiple breath wash out procedure in 16 asthmatic and 10 healthy children before and after exercise When compared to conventional spirometric variables VTG was the most sensitive test for detection of exercise-induced asthma (EIA) The VTG was significantly higher before exercise in the asthmatic children and increased significantly after exercise while it did not change in the healthy controls The significance of changes caused by EIA increased if VTG/TLC% or VTG/VC% were used Salbutamol inhalation normalized the VTG in all the asthmatic children

KEY WORDS Bronchial asthma bronchospasm exercise induced asthma exercise physiology salbutamol school-children volume of trapped gas

Bronchospasm is induced by exercise in about 90% of asthmatic children (8) Studies using peak expiratory flow rate (PEFR) and forced expiratory volume in one second (FEV_1) have shown that exercise has two distinct but opposite effects on lung function depending upon its duration Exercise of short duration (one to two minutes) does not cause bronchospasm but bronchodilatation Prolonged exercise (≥6 min) may produce bronchospasm which starts during the work but usually becomes maximal three to five minutes thereafter (1)

The type of exercise is important With the same work load running provokes more bronchospasm than cycling or kayaking while swimming causes very little (7 9)

It has been shown recently that an increase in the volume of trapped gas (VTG) is a sensitive indication of bronchospasm in provoked asthma (3)

The purpose of the present study was to assess different lung function tests for their ability to disclose exercise induced asthma (EIA) Inhaled salbutamol and a second short period of running was used to get the children as free from bronchospasm as possible (9) and thus to disclose the degree of abnormality at the start of the study

MATERIAL

Sixteen children 9–16 years old consecutively coming to the outpatient ward with a history of EIA took part in the study (Table 1) They were divided into three groups depending upon the severity of their EIA judged by story

Group A These two children had minimal asthma even after extended and heavy exercise Patient 1 was an elite tennis player and patient 2 was a competition swimmer They trained regularly and took disodium cromoglicate (DSCG) only during the pollen season

Group B comprised seven children all having slight but obvious EIA They took part in athletics at school and they were very seldom absent from these lessons Three of them took DSCG 20 mg×4 regularly (at 7 and 11 a m and at 3 and 7 p m)

Table 1 *Physical characteristics and case histories from 16 children with EIA*

Lomudal®=disodium cromoglicate Treated=+ not treated=− A B C=grouping of patients as described under Material

	Patient No	Sex	Age (y)	Height cm/ weight kg	Duration of asthma (y)	Allergy to	Other allergic symptoms
Group A	1	f	12	169/67	3	Animals pollen	Eczema rhinitis
	2	m	15	175/58	7	Dust moulds pollen	Rhinitis
Group B	3	m	9	127/25	7	Animals dust moulds	0
	4	f	9	140/27	8	Animals dust	0
	5	m	10	138/29	6	Animals moulds	Eczema rhinoco
	6	m	11	154/43	3	Pollen	Rhinitis
	7	m	13	149/40	10	Animals dust moulds	Rhinitis
	8	m	14	171/49	13	Dust moulds pollen	Eczema rhinocon
	9	m	14	169/56	12	Animals dust moulds pollen food	Eczema rhinoconj
Group C	10	m	11	151/43	10	Animals pollen food	Rhinitis
	11	m	12	145/33	10	Animals dust pollen	Rhinitis
	12	f	13	174/64	12	Animals dust pollen	Eczema rhinitis
	13	f	13	156/41	13	Animals dust moulds	Quincke s oedema
	14	f	14	157/46	9	Pollen	0
	15	m	14	175/60	7	Pollen	Eczema
	16	f	16	161/41	13	Pollen	Eczema

Group C comprised seven children with severe EIA They participated in school athletics except for prolonged running but were frequently absent due to their disease Five of them took DSCG 20 mg×4 regularly

The control material comprised ten healthy children 9–16 years old who were examined in the same way as the asthmatic children

Height (range 127–175 and 128–175 cm respectively) weight (range 25–67 and 25–66 kg respectively) and age were not statistically different in the asthmatic and healthy group

METHODS

The investigation was made during the winter when the subjects had no symptoms of allergic asthmatic disease or of any other active disease Informed consent to take part in the study was given by the parents and children All studies were performed in the afternoon between 1 and 3 p m DSCG was taken in the morning but not in the last five hours before the examination No other medication was allowed The children had neither subjective nor stethoscopical signs of bronchospasm before the examination Static and dynamic spirometry was performed in the sitting position with a Bernstein type spirometer With the subject breathing tidal volume breaths the nitrogen wash out was continued until the end expiratory nitrogen reached 2% (2) using an Ohio 700 nitrogen analyzer (Ohio Houston Texas) The subject then made a maximal inhalation a stopcock was turned and he rebreathed for five maximal breaths into a plastic bag by which time the concentration of nitrogen usually reached a plateau The nitrogen thus mobilized was defined as the volume of trapped gas (VTG) when expressed as air (13)

$$VTG = \frac{TLC \times FN_2ER - FRC \times 0\ 02}{0\ 80}$$

$FN_2ER = N_2$ fraction at the end of the rebreathing period FRC=functional residual capacity TLC=total lung capacity The wash out volume (WOV) functional residual capacity (FRC) and lung clearance index (LCI)= WOV/FRC were also calculated from the nitrogen wash out down to 2%

Student s *t* test for paired observations was used for the statistical analyses of the changes between the different experimental situations and for the comparison between healthy and asthmatic children

EXPERIMENTAL PROTOCOL

The first measurements were obtained when the children had rested for about 30 min beginning with the nitrogen wash out The children thereafter ran for 6 min on a treadmill with 5 degrees elevation at a speed of 6–8 km/hour aiming at a heart rate of 170 beats/min All measurements were repeated 5–12 min after exercise The children then inhaled two puffs of a dose aerosol (200 μg) salbutamol and 10 min later all measurements were repeated As soon as possible after another minute of running at the same workload the measurements were repeated once more

Hyposens therapy	Continuous Lomudal® treatment
+	–
+	–
+	+
–	+
+	–
–	–
+	–
+	+
+	–
–	+
+	+
+	+
+	–
+	+
+	–
+	+

RESULTS

As an illustration to the pronounced change in appearance of the rebreathing N_2 curves Fig 1 shows the end of the nitrogen wash out curves and the VTG rebreathing before and after exercise in a subject showing a pronounced reaction to exercise Notice the great increase in nitrogen concentration during each exhalation of VC in the rebreathing period[1] Fig 2 shows changes in some spirometric variables WOV and VTG after exercise in the same patient

The results from the whole material are given in Table 2 which shows the mean values and SD of WOV VTG and spirometric variables in the asthmatic as well as in the healthy children There was no consistent difference between the reaction to exercise among the asthmatic children in groups A and B Both children in group A had a slight reaction while in group B one did not react (no 7) one showed only a slight increase in VTG (no 5) and the rest suffered from moderate asthma The changes in group C were slightly more pronounced The difference between the groups was however not significant so the values of the asthmatic children are presented pooled as mean values (complete tables can be obtained on request from the authors) The patients taking DSCG did not differ in their reaction to exercise stress from those not taking the drug

Comparison between the healthy and diseased children

The VTG and derived variables of the healthy and asthmatic group were significantly different in all the experimental situations with the exception of the values obtained after the second short period of running

LCI was as sensitive as VTG/TLC % for detecting abnormalities after exercise but demonstrated no difference between the healthy

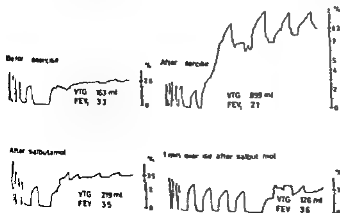


Fig 1 The end of the nitrogen wash out curves and VTG rebreathing in subject no 15 before and after 6 min of treadmill running 10 min after inhalation of 200 μg salbutamol and then after one minute of treadmill running VTG=volume of trapped gas FEV =forced expiratory volume in one second Kymograph speed before rebreathing 4 cm/min and during rebreathing 0 cm/min The N concentration in the rebreathing bag is marked on top of each scale

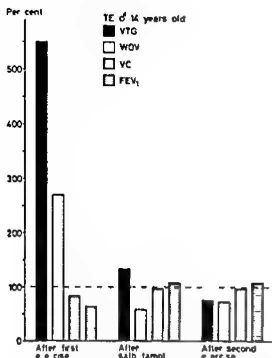


Fig 2 Percental changes in some spirometric variables WOV and VTG in the same boy as in Fig 1 The values before exercise were set to 100% VTG=volume of trapped gas WOV=wash out volume VC=vital capacity FEV_1=forced expiratory volume in one second

and diseased children before exercise The WOV showed the same trend but slightly less pronounced

FEV_1 was significantly lower ($p<0.05$) in the asthmatic children than in the healthy after exercise but not before exercise FEV% on the other hand separated diseased from healthy children both before and after exercise However it did not decrease significantly after exercise and was hence not so sensitive for showing EIA

Changes induced by exercise and salbutamol

(1) *Asthmatic children After first exercise (AE)* Children no 5 and no 7 showed no significant changes (less than 2 errors of a single determination) All the rest showed significant or highly significant changes in VTG VTG/TLC% VTG/VC% LCI and WOV FEV_1 and FEV% showed no significant changes

After inhalation of 200 µg salbutamol (AE+S) Compared to pre exercise values only FEV_1 showed a probably significant increase

After short exercise (ASE) Significant and probably significant decreases were found in VTG VTG/TLC% and VTG/VC% FEV% did not change significantly in comparison to the value obtained after salbutamol

(2) *Healthy children* No significant changes were induced by the three challenges in the healthy children (AE AE+S and ASE)

DISCUSSION

EIA has been recognized since 1679 (1) and has created a great deal of interest during the last years (1 20)

The purpose of this paper was to find a simple and sensitive test useful for detecting exercise induced asthma Running was preferred as exercise challenge as this has been shown to provoke asthma most consistently (10) Pulmonary function testing in asthmatic children is extensively discussed by Souhrada & Buckley (20) but no preference is given to any specific test According to Cropp & Schmultzler (5) changes in air conductance should be more sensitive than PEFR or maximum mid expiratory flow rate (MMEFR) and other common static or dynamic lung function variables On the other hand Godfrey & Konig (10) documented PEFR as clinically useful in a well planned study on the effects of different drugs on EIA

Thoracic gas volume (TGV) measured with a body plethysmograph includes all the gas in the chest at the end of a normal tidal volume expiration In the normal lung TGV is equal to functional residual capacity (FRC) as measured by helium dilution (14 20) Woolcock et al found TGV frequently higher than FRC in patients with asthma (21) The difference between these gas volumes has been referred to as trapped air volume (TAV) (14 21) Souhrada & Buckley (20) did not consider TAV sensitive or easily applicable TGV and hence also TAV is difficult to measure with high accuracy and Lovejoy et al (14) found both increases and decreases in TAV during pro-

Table 2. *Mean values and S.D. of conventional spirometric values, nitrogen wash out variables, VTG and derived variables*

BE=before exercise. AE=after exercise. AE+S=after 6 min of treadmill running followed by inhalation of 200 μg salbutamol. ASE=after 6 min of treadmill running followed by inhalation of 200 μg salbutamol and another minute of running. VTG=volume of trapped gas. TLC=total lung capacity. VC=vital capacity. FEV_1=forced expiratory volume in one second. FEV%=FEV_1/VC. FRC=functional residual capacity. RV=residual volume. LCI=lung clearance index. WOV=wash out volume. The significance of the changes in the asthmatic subjects between values obtained at rest and during different experimental conditions is shown with asterisks below asthmatic children, while differences between the healthy children and the asthmatic children under identical experimental conditions are shown with asterisks below healthy children. Within the healthy group of children no statistically significant changes occurred.

		Asthmatic children n=16				Healthy children n=10			
		BE	AE	AE+S	ASE	BE	AE	AE+S	ASE
VTG ml	M	109	234	103	87	70	73	69	73
	S.D.	36	193	47	21	19	16	16	24
VTG/TLC%	M	2.7	5.9	2.4	2.0 *	1.9	1.9	1.8	1.9
	S.D.	0.8	2.5	0.7	0.4	0.4	0.3	0.4	0.3
VTG/VC%	M	3.6	9.4	3.1	2.5	2.3	2.4	2.3	2.4
	S.D.	1.3	5.7	1.0	0.5	0.5	0.4	0.6	0.4
VC l	M	3.12	2.85	3.25	3.27	3.12	3.17	3.15	3.11
	S.D.	0.70	0.60	0.64	0.62	0.93	0.95	0.94	0.95
FEV_1 l	M	2.33	2.00	2.62	2.68	2.64	2.64	2.65	2.69
	S.D.	0.51	0.47	0.53	0.54	0.91	0.92	0.90	0.89
FEV %	M	74.8	70.9	80.3	81.9	83.7	84.2	83.3	86.1
	S.D.	7.5	10.0	6.1	8.0	7.1	8.3	7.4	6.0
TLC l	M	4.1	4.3	4.1	4.1	3.9	3.9	3.9	4.0
	S.D.	1.2	1.7	1.0	0.9	1.1	1.1	1.0	1.2
FRC l	M	1.9	2.3	1.7	1.7	1.7	1.8	1.7	1.8
	S.D.	0.7	1.5	0.5	0.4	0.6	0.5	0.5	0.5
RV l	M	0.9	1.4	0.8	0.9	0.7	0.8	0.7	0.8
	S.D.	0.6	1.4	0.4	0.3	0.4	0.2	0.2	0.3
LCI	M	8.4	12.5 *	8.9	8.7	8.0	7.9	8.1	7.8
	S.D.	1.0	3.4	1.4	1.4	0.9	0.7	0.7	0.8
WOV l	M	15.4	28.3	15.1	14.7	13.5	14.0	14.0	13.6
	S.D.	5.4	16.1	5.1	4.7	4.5	4.3	3.9	4.1

*$p<0.05$=probably significant
**$p<0.01$=significant
***$p<0.001$=highly significant

voked bronchospasm. In contrast to TAV, VTG as measured by us includes only trapped gas which can be mobilized during maximal breaths. VTG might thus seem less well defined than TAV. On the other hand, with a good technique the measured values are well reproducible. The error of a single determination was 12 ml (3). It has also been shown that five maximal breaths give a good estimation of the nitrogen content in the lung measured at the beginning of the nitrogen wash out (18). The same procedure at the end of the nitrogen wash out should be equally effective. In our experience very little further nitrogen is released by prolonging the rebreathing further, which also is tiresome for the subject. In our healthy controls VTG showed a high positive relation to VC and TLC. We consider a VTG/TLC% above 2.7% (mean +2 S.D.) (Table 2) at rest abnormal in children. After exercise it decreased in the healthy controls and a value of VTG/TLC% above 2.5% (mean +2 S.D.) might be considered abnormal. For a normal standard

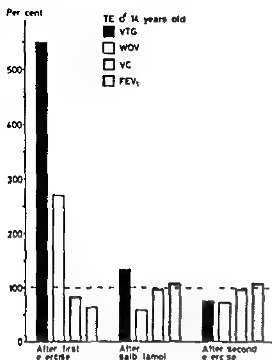


Fig 2 Percental changes in some spirometric variables WOV and VTG in the same boy as in Fig 1 The values before exercise were set to 100% VTG=volume of trapped gas WOV=wash out volume VC=vital capacity FEV_1=forced expiratory volume in one second

and diseased children before exercise The WOV showed the same trend but slightly less pronounced

FEV_1 was significantly lower ($p<0.05$) in the asthmatic children than in the healthy after exercise but not before exercise FEV% on the other hand separated diseased from healthy children both before and after exercise However it did not decrease significantly after exercise and was hence not so sensitive for showing EIA

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Table ? *Mean values and S D of conventional spirometric values nitrogen wash out variables VTG and derived variables*

BE=before exercise AE=after exercise AE+S=after 6 min of treadmill running followed by inhalation of 200 μg salbutamol ASE after 6 min of treadmill running followed by inhalation of 200 μg salbutamol and another minute of running VTG=volume of trapped gas TLC=total lung capacity VC=vital capacity FEV_1=forced expiratory volume in one second FEV%=FEV_1/VC FRC=functional residual capacity RV=residual volume LCI=lung clear an e index WOV=wash out volume The significance of the changes in the athmatic subjects between values obtained ■ rest and during different experimental conditions is shown with asterisks below "asthmatic children" while differences between the healthy children and the asthmatic children under identical experimental conditions are shown with asterisks below healthy children Within the healthy group of children no statistically significant changes occurred

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		BE	AE	AE+S	ASE	BE	AE	AE+S	ASE
VTG ml	M	109	271	103	8?	70	73	69	73
	S D	36	193	47	21	19	16	16	24
VTG/TLC%	M	2 7	5 9 *	? 4	2 0	1 9	1 9	1 8	1 9
	S D	0 8	2 5	0 7	0 4	0 4	0 3	0 4	0 3
VTG/VC%	M	3 6	9 4	3 1	? 5	2 3	? 4	2 3	2 4
	S D	1 3	5 2	1 0	0 5	0 5	0 4	0 6	0 4
VC l	M	3 1?	? 85	3 ?5	3 ?7	3 12	3 1?	3 15	3 11
	S D	0 70	0 60	0 64	0 6?	0 93	0 95	0 94	0 95
FEV 1	M	2 33	? 00	? 6?	2 68	2 64	2 64	2 65	2 69
	S D	0 51	0 42	0 53	0 54	0 91	0 92	0 90	0 89
FEV %	M	74 8	70 9	80 3	81 9	83 7	84 2	83 3	86 1
	S D	7 5	10 0	6 1	8 0	7 1	8 3	7 4	6 0
TLC l	M	4 1	4 3	4 1	4 1	3 9	3 9	3 9	4 0
	S D	1 2	1 7	1 0	0 9	1 1	1 1	1 0	1 2
FRC l	M	1 9	2 3	1 7	1 7	1 7	1 8	1 7	1 8
	S D	0 7	1 5	0 5	0 4	0 6	0 5	0 5	0 5
RV l	M	0 9	1 4	0 8	0 9	0 7	0 8	0 7	0 8
	S D	0 6	1 4	0 4	0 3	0 4	0 2	0 2	0 3
LCI	M	8 4	1? 5	8 9	8 7	8 0	7 9	8 1	7 8
	S D	1 0	3 4	1 4	1 4	0 9	0 7	0 7	0 8
WOV l	M	15 4	?8 3	15 1	14 7	13 5	14 0	14 0	13 6
	S D	5 4	16 1	5 1	4 7	4 5	4 3	3 9	4 1

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this material however, is too small and more extensive data have been collected (Svenonius et al to be published)

All the examined asthmatic children with anamnestic EIA increased their VTG after 6 minutes running on a treadmill with the exception of two boys (nos 5 and 7) These two boys did not change in the other spirometric variables either They were exhausted after the running Running is known to be the most effective form for provoking EIA (9) Hence these subjects probably suffered their anamnestic asthma not because of exercise but because of allergens in the gymnasium

After inhalation of salbutamol VTG decreased in all the reacting children and showed a further decrease after another minute of running Whether this second decrease was due to the short running or to the salbutamol could not be resolved by our experimental protocol The healthy children showed no changes in any variables after bronchodilatation or after the second exercise test

Lung clearance index and wash out volume were also found sensitive for demonstrating provoked asthma confirming the report of Colldahl & Lundin (4) However VTG disclosed bronchospasm better than the lung clearance index in the pre exercise test The only variable besides VTG showing an abnormality in the children before the first running was FEV% However this test like FEV_1 did not disclose significant changes in the asthmatic children after running The VTG measurements were found less exhausting than FEV_1 and did not provoke asthma which the FEV_1 can (11)

It has been discussed whether asthma primarily affects large or small airways Small airways with a diameter of less than 2 mm contribute to only about 20% of the airflow resistance in the tracheobronchial tree Up to 50% of these airways could be occluded and still probably pass undetected by conventional measurements of lung function including the FEV_1 (15) Acute asthma must affect also large airways as FEV_1 often is decreased in that disease and FEV_1 mainly is sensitive to changes in large airways (19) Two reasons thus speak in favour of the hypothesis that airways closure causing VTG should mainly be confined to small airways (1) that VTG increased in all reacting children while FEV_1 did not change in five of the reacting ones, and (2) that small airways should be more easily closed than large

Hence our results seem to be contradictory to those of Mildon et al (17) who considered EIA confined to large airways However they did not apply any test specific for small airways disease We conclude that EIA in the early stage is confined only to small airways but that larger airways will be engaged as the bronchospasm becomes more pronounced Subjective and/or auscultatory bronchospasm was seen mainly in children with significant decrease in FEV_1 after exercise It must be stressed that decrease in FEV_1 did not occur in any child who did not also show pronounced increase of VTG Uneven gas distribution indicates airways disease (12) Increase in LCI was related to increase in VTG and high VTG was always combined with a very sloping nitrogen alveolar plateau during each exhalation of VC (Fig 1 After exercise) This slope probably represents uneven emptying of regions with partly closed off alveoli

We did not attempt to quantify small airways involvement with frequency dependent compliance (22) or closing volume (16) as these techniques are difficult to apply on children and the latter test seems to give contradictory results in provoked asthma (6) To summarize the VTG test was easy to apply in children The airway constriction shown by the VTG test is probably confined to small airways and occurred in all reacting children (14/16) Five of them did not disclose large airways constriction as measured by FEV_1 Thus VTG disclosed significant airway constriction in some children in whom subjective or objective signs of bronchoconstriction could not be detected by conventional spirometric methods

ACKNOWLEDGEMENTS

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URINARY TRACT INFECTIONS CAUSED BY *PROTEUS MIRABILIS* IN CHILDREN

The Antibody Response to O and H Antigens and Tamm Horsfall Protein and Bacterial Adherence to Uro epithelium

P LARSSON A FASTH U JODAL A SOHL ÅKERLUND
and C SVANBORG EDÉN

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ABSTRACT Larsson P Fasth A Jodal U Sohl Åkerlund A and Svanborg Eden C (Department of Immunology Institute of Medical Microbiology and Department of Paediatrics University of Goteborg Goteborg Sweden) Urinary tract infections caused by *Proteus mirabilis* in children The antibody response to O and H antigens and Tamm Horsfall protein and bacterial adherence to uroepithelium Acta Paediatr Scand 67 591 1978.—Sera from seven girls with acute symptomatic pyelonephritis and nine children with acute symptomatic cystitis caused by *Proteus mirabilis* were analysed for antibodies against the bacterial O and HI antigens and the Tamm Horsfall protein An increase in antibody levels against O antigen and Tamm Horsfall protein was noted only in patients with acute pyelonephritis indicating that antibody determinations can be useful in differentiating between upper and lower urinary tract infection caused by *Proteus* in similarity to those caused by *E coli* In contrast no difference in adhesive ability was noted comparing *Proteus* strains causing acute pyelonephritis or cystitis

KEY WORDS Urinary tract infections serum antibodies Proteus immunology Tamm Horsfall protein bacterial adherence children

E coli is the most common bacterial cause of urinary tract infection (UTI) in children The second most common species is *Proteus* which is especially prevalent in boys after one year of age (24) and in patients with neurogenic bladder disorders (5)

Determination of antibodies against the O antigen of the infecting *E coli* by indirect hemagglutination (18) or by the more sensitive enzyme linked immunosorbent assay (ELISA) (6) can be useful in differentiating acute pyelonephritis from acute cystitis (2 11 12 13) Quantitation of auto antibodies to Tamm Horsfall protein has been shown to differentiate between upper and lower UTI (9)

To compare the host–parasite relationship in UTI caused by *E coli* and *Proteus* we have studied the humoral antibody response to *Proteus* O and H antigens and to the Tamm Horsfall protein in children with acute pyelonephritis and acute cystitis caused by *P mirabilis* This is the most frequent species in the serological system for *Proteus* of Kauffmann & Perch (14)

In UTI due to *E coli* ability in attaching to human uroepithelial cells in vitro correlates with virulence (22 23) The *Proteus* strains were tested for adhesive properties

PATIENTS AND METHODS

Patients

Seven girls aged 1½–8 years with acute pyelonephritis and 9 patients (6 boys and 3 girls) age ?–11 years with acute cystitis were studied All had bacteriuria of at least 100 000 *P mirabilis* per ml The criteria for pyelonephritis were fever exceeding 38 5 C and two or more of the following micro-sedimentation rate (MSR) >25 mm/h C reactive protein (CRP) >20 mg/l and transiently decreased renal concentrating capacity (<815 mosmol/l) Criteria for cystitis were symptoms of burning and frequency but absence of loin pain temperature not exceeding 38 C and normal laboratory findings (13) The clinical and laboratory data are shown in Table 1 Results of intravenous pyelography and micturition cysto-urethrography performed on most of the patients are also given

The serum samples were stored at −20°C until analysed

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PATIENTS AND METHODS

Patients

Seven girls aged 1½–8 years with acute pyelonephritis and 9 patients (6 boys and 3 girls) age 2–11 years with acute cystitis were studied All had bacteriuria of at least 100 000 *P mirabilis* per ml The criteria for pyelonephritis were fever exceeding 38 5 C and two or more of the following micro-sedimentation rate (MSR) >25 mm/h C reactive protein (CRP) > 0 mg/l and transiently decreased renal concentrating capacity (<815 mosmol/l) Criteria for cystitis were symptoms of burning and frequency but absence of loin pain temperature not exceeding 38 C and normal laboratory findings (13) The clinical and laboratory data are shown in Table I Results of intravenous pyelography and micturition cysto-urethrography performed on most of the patients are also given

The serum samples were stored at −20°C until analysed

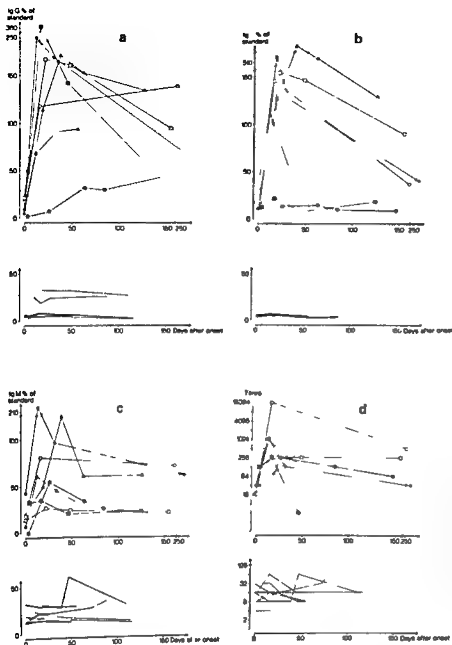


Fig 1 Proteus O antibodies in serum during acute pyelonephritis (*top*) and cystitis (*bottom*) Antibodies measured with the ELISA of (from left to right) (*a*) IgG (*b*) IgA and (*c*) IgM classes (*d*) Antibodies measured with the indirect hemagglutination method Each line represents one patient O=patient J W ●=A L □=M M ■=M O Δ=A K ▲=K K *=M C

O and H serotyping

Biochemical characterization and O and H serotyping were performed as earlier described (15) Antisera against some O antigens outside the 01-49 system of Kauffmann & Perch were available (15) These provisional O groups were denoted OA–E Antisera against the most common H types 1 2 3 and 4 were also used (16)

Adhesion testing

The method has been described in a separate paper (21) Briefly human urinary tract epithelial cells obtained from the sediment of fresh morning urine from a healthy person were suspended in phosphate buffered saline (PBS) and quantitated by direct light microscopy using a Burker chamber Bacteria suspended in buffer were quantitated similarly 10^9 bacteria and PBS were added to 10^5 epithelial cells to a volume of 1 ml The mixture was incubated and slowly rotated at 37 C for 60 min Unattached bacteria were eliminated by repeated washing and stained dead epithelial cells were excluded by the addition of a drop of trypan blue to the cell suspension Bacteria adhering to each of 40 epithelial cells were calculated using the Burker chamber

Antibody determinations

O antibodies were determined with the indirect hemagglutination method (11) as well as the enzyme linked immunosorbent assay (ELISA) (12) The antigens used were prepared from the strains isolated from the patients

When the infecting strain had an H1 antigen antibodies against this antigen were determined using the ELISA technique The H1 antigen was prepared from a *Proteus* 07H1 strain (Kauffmann–Perch designation F27) according to Ada et al (1) and used in a concentration of 0 1 g/l in the ELISA

Antibodies to the Tamm Horsfall protein were assayed

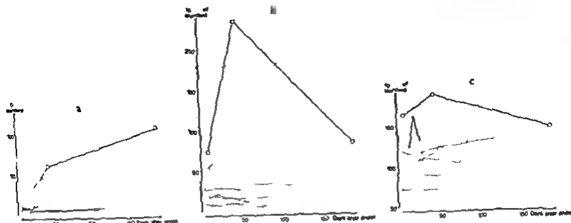


Fig 2 Proteus H1 antibodies in serum during acute UTI Antibodies measured with the ELISA of (from left to right) (*a*) IgG (*b*) IgA and (*c*) IgM classes Dotted lines indicate patient J W with pyelonephritis Full lines represent patients with cystitis

with the ELISA technique as earlier described (9) All antibody values obtained with the ELISA were expressed in per cent of standard sera

Because of small serum volumes available all samples could not be analysed with all tests and reduction of sera with β mercaptoethanol for the indirect hemagglutination testing had to be omitted

To compare the mean adherences statistically the Wilcoxon rank sum test was used (3)

RESULTS

Serum antibody response to Proteus O and H antigens

During the course of infection an increase was noted in O antibody levels of IgG IgA and IgM classes as determined with the ELISA in patients with acute pyelonephritis but not in those with cystitis (Fig 1*a–c*) One patient with pyelonephritis (A L) showed only a slight increase in O antibodies of the IgG class no rise in IgA antibodies but in IgM antibodies an evident response was noted Another patient with pyelonephritis (M O) developed very high levels of IgG antibodies during the infection whereas the levels of IgA and IgM remained unaffected The differing serological response of these two patients could not be correlated to the course of the infection

Using the indirect hemagglutination method for measurement of O antibodies an increase in titres was also shown in patients with pyelonephritis but not with cystitis (Fig 1*d*)

The H1 antibody levels remained unchanged during the infection in all patients with cystitis (Fig 2*a–c*) The only patient with pyelonephritis (J W) with an H1 containing strain showed an increase in IgA antibody levels to H1 A similar tendency was noted in IgG antibody amounts while the IgM level remained unchanged

Serum antibody response to Tamm Horsfall protein

Three out of 5 patients with pyelonephritis showed a significant change in antibody levels to the Tamm Horsfall protein of the IgG class (Fig 3*a*) For the IgA and IgM antibodies to the protein as well as for the antibodies of all three immunoglobulin classes in children with cystitis however no consistent change was found (Fig 3*b–c*)

Adhesion of P mirabilis strains to uro-epithelial cells

Four out of seven pyelonephritis strains (mean 11 bact /cell) and six out of nine cystitis strains (mean 14 bact /cell) adhered with >10 bacteria per cell Adhesion was irregular however and high mean adhesion values were due to large numbers of bacteria adhering to few epithelial cells rather than uniform spread of bacteria on most cells Statistically no significant dif

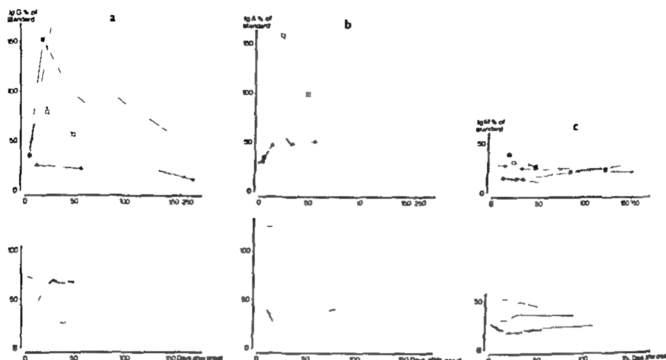


Fig 3 Serum antibodies to Tamm Horsfall protein during acute pyelonephritis (*top*) and cystitis (*bottom*) caused by *Proteus* Antibodies measured with the ELISA of (from left to right) (*a*) IgG (*b*) IgA and (*c*) IgM classes Symbols as in Fig 1

Table 1 *Clinical and laboratory data of children with acute pyelonephritis or cystitis caused by Proteus mirabilis*

P×4=4 episodes of acute pyelonephritis C×1=1 episode of acute cystitis Red r 2–3=parenchymal reduction+reflux grade 2 or 3 E=provisional O serogroup Na=non agglutinating strain NT=non typable H antigen=1 2 3 4 ND=not done 13 30=strain giving strong agglutination with anti 013 and 030 antisera

Patient	Age (yrs at onset)	Previous UTI	MSR (mm/h)	CRP (mg/l)	Concentrating capacity (mOsmol/l)	Radiological findings	Proteus serotype O	Proteus serotype H	Adhesion (mean no of bacteria/epithelial cell)
Acute pyelonephritis									
A K ♀	1½	P×4	57	22	675	Red r3	40	4	18
A L ♀	3	P×1	22	97	700	Red r 3	Na	NT	0
M O ♀	3	O	34	36	690	Normal	29	NT	0
K K ♀	5	P×4	ND	64	734	Red r 2	28	2	11
J W ♀	4½	O	39	133	ND	Red	Na	1	14
M C ♀	7	P×6	45	41	ND	Red	Na	NT	0
M M ♀	8	P×1	11	31	770	r 2	Na	NT	32
Acute cystitis									
J D ♀	2	C×1	21	<1	ND	Normal	E	1	19
J O ♂	3	O	23	<1	1 250	Normal	10	1	25
A I ♀	3	O	16	<1	820	Normal	10	1	2
A D ♂	4	O	7	<1	1 133	Normal	13 30	1	31
M E ♂	4	O	25	1–2	944	ND	Na	1	8
F L ♂	4½	O	20	<1	1 100	ND	23	1	6
J H ♀	4½	C×1	4	<1	1 035	Normal	Na	1	10
P J ♂	8	C×1	6	<1	1 479	Normal	Na	1	16
B K ♂	11	O	7	<1	762	Normal	10	1	12

ference in adhesive properties was found between strains isolated from the patients with acute pyelonephritis and acute cystitis (Table 1)

DISCUSSION

Most cases of acute pyelonephritis in children are caused by *E coli* Excluding patients with obstructions and neurogenic bladder disorders only 1–2% of all children with pyelonephritis have *Proteus* as the causing microorganism (Jodal unpublished) Recent studies have shown however that *Proteus* is a common cause of UTI in boys (4 8) In a study by Winberg et al (24) renal scarring was found in 13% of the boys and 4 5% of the girls prospectively followed from their first recognized symptomatic UTI mostly caused by *E coli* This difference in frequency could have many reasons but in the present work all children with pyelonephritis due to *Proteus* were girls and 5 out of 7 showed renal parenchymal reduction This suggests that children with acute pyelonephritis caused by *Proteus* should be thoroughly investigated In women aged 8–80 years Fairley et al (7) showed that *Proteus* infections in the urinary tract were of renal rather than bladder origin as determined by clinical data and the bladder wash out technique

The ability to attach to human uroepithelial cells *in vitro* is high for *E coli* bacteria isolated from the urine of patients with acute pyelonephritis intermediate for acute cystitis low for asymptomatic bacteriuria and lowest for normal faecal *E coli* (23) The adhesive ability of the *Proteus* strains tested showed no correlation to clinical origin The mean number of adhering bacteria per epithelial cell was generally lower for *Proteus* than for *E coli* strains causing symptomatic UTI (23) No definite conclusions concerning the role of adhesion in *Proteus* causing UTI can be drawn from the few strains tested so far but a larger material of *Proteus* strains is presently being investigated

This paper shows that O antibody levels increase during the course of an acute attack of pyelonephritis caused by a *P mirabilis* strain and that the O antibody levels in patients with cystitis remain unaffected just as earlier found in patients with *E coli* infections (2 11) However the antibody response to the O antigen in 2 of the patients with pyelonephritis was not very pronounced This may be due to differences in the antigenic mass presented to the host Studies on the immune response to the somatic antigen of *P mirabilis* in rats have shown that certain strains induce synthesis of serum IgM antibodies whereas other strains give a rapid appearance of IgG antibodies (20) If these observations are valid also in man an irregularity in the serum O antibody response may be accounted for in some patients

There was also an indication in the present work that the antibody levels to the *Proteus* H1 antigen may rise in patients with acute pyelonephritis This is supported by studies on experimental ascending pyelonephritis caused by *P mirabilis* in rats showing the appearance of immobilizing serum antibodies during the development of infection (19) These antibodies considered to be directed against the bacterial flagella were early in the course of pyelonephritis associated with both 19S and 7S globulins and later only with 7S proteins To our knowledge there are no data available on the appearance of flagellar (H) antibodies in acute UTI caused by *E coli*

A rise in serum IgG antibody levels but not of IgM antibodies to the Tamm Horsfall protein has earlier been reported in patients with acute pyelonephritis caused by *E coli* (9) The present work shows that acute pyelonephritis caused by *P mirabilis* can also increase such antibodies The Tamm Horsfall protein can be demonstrated in the tubuli of the kidney (17) and the appearance of serum antibodies to this protein might be the result of tissue damage in this region of the kidney Another possible mechanism might be cross reactions between bacterial antigens and renal structures (10) This would then obviously re

quire a cross reaction between renal tissue and most pyelonephritis inducing *E coli* and *P mirabilis* since an anti Tamm Horsfall anti body response is so consistently found in the patients with acute pyelonephritis (9) This seems unlikely and is not supported by our unpublished search for cross reactions be tween bacteria and the Tamm Horsfall pro tein

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LONG TERM PROPHYLAXIS WITH METHENAMINE HIPPURATE IN GIRLS WITH RECURRENT URINARY TRACT INFECTIONS

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ABSTRACT Petersen S (Department of Paediatrics Glostrup Hospital Glostrup Denmark) Long term prophylaxis with methenamine hippurate in girls with recurrent urinary tract infections Acta Paediatr Scand 67 597 1978 —Twenty girls aged 5–12 years with recurrent urinary tract infections were treated with methenamine hippurate Hiprex® for a period of 12 months The number of infections per patient per year was reduced from 3 1 to 0 7 ($p<0\ 001$) After prophylaxis was stopped this number increased to 1 4 which is a significantly higher incidence than occurred during treatment ($p<0\ 05$) A few girls complained of the taste but no side-effects were observed Methenamine hippurate is a useful alternative in long term prophylaxis against recurrent urinary tract infections in girls

KEY WORDS Urinary tract infections methenamine hippurate

A urinary tract infection will appear in 3% of girls and 40% of these will have one or more recurrences (11) Long term treatment with sulphonamides nitrofurantoin or trimethoprim sulphamethoxazole has shown good results in preventing recurrences (8 10) but allergic reactions or bacterial resistance make alternative treatment desirable

The aim of this investigation was to study the long term prophylaxis of urinary tract infections with methenamine hippurate Hiprex® in girls with a pronounced tendency to recurrent urinary tract infections

PHARMACOLOGY

Methenamine hippurate is the salt of methenamine and hippuric acid It is easily absorbed from the gastrointestinal tract and is rapidly excreted in the urine The two substances dissociate in the urine to form hippuric acid and methenamine Hippuric acid exerts its bacteriostatic effect through acidification of the urine Methenamine has no antibacterial effect but if the urine is acid it is hydrolysed and releases formaldehyde which is bacteriostatic

MATERIAL AND METHODS

Twenty-one girls mean age 9 years range 5–12 years with recurrent urinary tract infections were included in the study They were selected for the investigation if they fulfilled the following criteria 1) age more than 5 years 2) normal glomerular filtration rate and 3) at least three infections within 12 months or two infections within six months prior to the study Fourteen had vesicoureteral reflux and four had chronic pyelonephritis

The girls were followed in the out patient clinic before during and after treatment and urinary cultures were performed monthly using the dip-slide technique (Uricult®) Quantitative cultures of mid stream urine samples were performed if bacterial growth was found on Uricult® or if the patient had symptoms of urinary tract infection The lower limit for significant bacteriuria was placed at 10^5 bacteria/ml When significant bacteriuria was found Hiprex® was replaced by chemotherapeutic agents according to a sensitivity test Chemotherapy was continued for 10 days and if the urine was sterile Hiprex® was resumed If bacteriuria recurred within three months a low dose chemotherapeutic treatment was given for three months whereupon Hiprex® was resumed

The girls received 0 5 g methenamine hippurate morning and evening The mean duration of the treatment was 12 months Before the period of Hiprex® treatment the patients had been observed for 13 months and after the period of Hiprex® treatment they were observed for an other period of 12 months (Table 1)

The number of urinary tract infections per patient per year for each of the periods before during and after methenamine hippurate prophylaxis was calculated and a paired Student s t test was applied to the results

RESULTS

Of the 21 girls 20 completed this study One refused to continue after two months because she disliked the taste of the tablets

Table 1 *The number of urinary tract infections in the 20 patients during the study*

Period		Before treatment	During treatment	After treatment
Length of the period in months	Mean	13	12	12
	Range	5–24	9–13	4–18
Number of infections per patient per year	Mean	3 1	0 7	1 4
	Range	1 7–5 0	0 0–3 0	0 0–4 0

The difference between the number of infections before and during treatment is significant ($p<0\ 001$) The differen e between the number of infections during and after treatment is significant ($p<0\ 05$)

As seen in Table 1 the number of infections per patient per year before treatment was 3 1 During treatment the number was reduced to 0 7 The difference is highly significant ($p<0\ 001$) After the period of treatment the number of infections increased to 1 4 which is a significantly higher ($p<0\ 05$) incidence than that which was found during treatment

Of the 20 girls 11 had no infections during Hiprex® treatment and in the remaining nine the number was reduced In eight of 11 girls without infections during treatment the infections recurred after treatment was stopped During the post treatment observation period 12 girls had more and four had fewer infections than during treatment while in the remaining four there was no difference

No cases of haematuria allergic reactions or gastrointestinal side effects were observed A few children complained of the taste but only one refused to take the drug

DISCUSSION

In the present investigation long term prophylaxis with methenamine hippurate resulted in a reduction of urinary tract infections to 23% of the number in the untreated period This result could have been influenced by the fact that in girls with urinary tract infections the risk of recurrence is decreased with increasing age (6) However the finding that the incidence of infection increased after treatment was stopped indicates that spontaneous recovery was not essential to the result The finding in this study that the number of infections was lower after one year of successfu prophylaxis than before prophylactic treat ment was instituted corresponds well with the fact that the risk of recurrence is decrease with increasing length of a period free of in fection (6)

In a comparative study of the long term prophylactic effect of nitrofurantoin methen amine hippurate trimethoprim and trimetho prim sulphamethoxazole Kasanen et al (5 found the effect of methenamine hippurate al most equal to that of nitrofurantoin but no as good as trimethoprim or trimethoprim sulphamethoxazole However the incidence of side effects in patients treated with methen amine hippurate was only half that observed i the nitrofurantoin or trimethoprim sulpha methoxazole groups

Methenamine hippurate has given the bes results in uncomplicated cases of persistent o recurrent urinary tract infections (1 2 3 7 9) In these cases 75–80% of the patients wer free from bacteriuria during the treatment I the presence of chronic pyelonephritis or cal culus the cure rate has been reported to b 40–60% (3 4)

Methenamine hippurate has also bee shown to be well tolerated in long term treat ment (3) Reported side effects are nausea vomiting pruritus allergic skin reactions an bladder irritation (haematuria) All side effect are reversible and occur infrequently (1 3) Following the treatment of 531 women durin pregnancy with methenamine hippurate (1) n

signs of foetal injury were observed On account of the acidification of the urine methenamine hippurate should not be given together with sulphonamides

The main indication for methenamine hippurate is long term prophylaxis in patients with chronic or recurrent urinary tract infections after treatment of the acute infection with chemotherapeutic agents or antibiotics

Essential advantages of methenamine hippurate in long term treatment is that development of bacterial resistance during treatment has not been reported and according to the rapid absorption and low blood concentration of methenamine hippurate are intestinal or mucous membrane flora not supposed to be affected

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CONGENITAL DEFECTS IN A COHORT FOLLOWED FOR SEVEN YEARS

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ABSTRACT Klemetti A (Third Department of Pathology University of Helsinki Helsinki and the Central Hospital of Middle Finland Jyvaskyla Finland) Congenital defects in a cohort followed for seven years Acta Paediatr Scand 67 601 1978 —A geographically limited cohort of Finnish children was followed from birth for seven years and all congenital abnormalities were recorded and classified and special attention was given to the cumulative detection rate and the time of detection of various defects Of 3 674 pregnancies 135 babies with or without defects were stillborn or died during the neonatal period The remaining 3 539 were followed up to seven years when the percentage follow up was 81 7% Detailed information on 76 malformed livebirths registered in the neonatal period was available in [illegible] cases (82 9%) The diagnosis was found to be incorrect in 6 cases and additional defects were registered in 7 of these children Additional congenital abnormalities detected in the follow up study were divided into three groups all congenital disorders or abnormalities with prenatal etiology (248 children) all congenital defects (111 of these 248) and structural malformations (31 of these 111) The cumulative detection rates in these groups increased with time and at the end of the study when the children were aged seven were 9 4% 5 6% and 2 6% respectively

KEY WORDS Congenital malformations follow up study

Efforts to compare the incidence of congenital malformations and its possible changes in different populations are hampered by several factors For example the figures are affected by lack of agreement on definitions and by varying detection and reporting rates To evaluate the impact of such factors on reporting of defects a cohort from a defined geographical area was followed for seven years and all congenital abnormalities were carefully recorded and classified Special attention was paid to the overall and cumulative detection rate and to the time of detection of various defects

MATERIAL

The material was collected from the province of Middle Finland in the period Nov 1 1963–Dec 31 1964 The population of Middle Finland is about 250 000 and the relatively stable rural population is about 170 000 Initially 3 6 4 women were included prior to or during the fifth month of pregnancy (5)

Of the children born to these women 103 (2 8%) had at least one malformation detected at birth or during the neonatal month About half of these developmental defects were disabling or lethal (5) and in addition to these the pregnancies of 108 women (2 9%) ended in a miscarriage between the 17th and 27th weeks or in a stillbirth or in death of the child in the neonatal period

THE FOLLOW UP STUDY

The follow up study was carried out in 1971–1972 when the average age of the children was seven years The children were identified through the Child Health Centres by name sex date of birth mother s name and address These Centres take over responsibility for health care when the child is 14 days old and the supervision is continued until the child is seven The children are examined by physicians and public health nurses about twice a year on average and all the information concerning hospital care operations etc is registered in the files of the Centre

Child Health Centres operate under the same law as the Maternity Health Centres so there is at least one in every locality About 97% of children less than one year old and 96% from one to six years are registered in these centres (10) The services are available free of charge

Table 1 *Additional congenital abnormalities detected in children with previously diagnosed malformations*

Earlier diagnosis	Later additional diagnosis
Mongolismus atresia intestini	Defectus septi atriorum
Cheilo-gnatho-urano-palatoschisis omphalocele	Defectus septi atriorum hernia ing
Dislocatio coxae cong	Uvula Bifida
Dislocatio coxae cong	Retentio testis
Dislocatio coxae cong	Torticollis musculorum strabismus
Dislocatio coxae cong	Strabismus
Hypospadia	Strabismus
Hypospadia	Convulsiones

Since there is only one central hospital with a pediatric ward in the province of Middle Finland it was very likely that the children with congenital defects requiring treatment would find their way to it The files of this hospital were therefore reviewed to provide further information about the children Visual and auditory screening studies in the district have provided additional data (11)

All the information obtained during the investigation was checked and collated by the author

DEFINITIONS AND NOMENCLATURE OF CONGENITAL DEFECTS

The congenital defects were grouped into three categories according to different definitions of the concept of a congenital malformation 1) Structural malformations 2) All congenital defects 3) All disorders or abnormalities with apparent prenatal etiology

Structural malformations included congenital abnormalities due to impaired organogenesis

All congenital defects were considered to include both generally accepted structural malformations and those developmental defects which may not necessarily reflect abnormal organogenesis and whose inclusion in the light of the literature is considerably variable thus causing variation in incidence figures These debated malformations were dislocation of the hip abdominal hernias pyloric stenosis undescended testis and malposition of feet

All disorders or abnormalities with prenatal etiology included both functional and morphological disorders (e g strabismus mental retardation dacryostenosis and phimosis) All the cases accepted to this group were diagnosed in the pediatric ward or the outpatient clinic of the hospital and confirmed by a specialist The final selection of the cases was done by the author and thus affected by subjective views

RESULTS

Completeness of the follow up

The original material was 3 674 pregnant mothers Of their children 135 with or without defects were stillborn or died during the neonatal period The remaining 3539 were followed for seven years though the final follow up percentage was 81 7 The vast majority of lost cases were recorded as having emigrated to Sweden during the follow up period Recorded data on visits to the paediatric ward were found in 938 cases (26 5%)

During the observation period 9 children died Of these one had a congenital heart malformation (ventricular septal defect) and two had malignant diseases (lymphatic leukaemia spongioblastoma) All of these were considered healthy in the original study

Life span of the defective children

All 76 children with immediately presenting all congenital defects but who survived the neonatal period were all alive at the age of 7 years More detailed information was available in only 63 cases (82 9%) Children with disabling malformations requiring treatment could easily be traced from the hospital whereas follow up was less certain for children with insignificant abnormalities The original diagnosis was correct in 57 cases (90 5%) Most of the errors occurred in diagnosing congenital dislocation of the hip and malposition of feet (four overdiagnoses and two misdiagnoses) Of seven additional children in whom congenital disorders were diagnosed late two had congenital heart disease (Table 1)

Classification of the defects

According to the classification system presented above the total number of children in the all disorders group was 248 Of these structural malformations were found in 31 cases (Table 2) and 28 had required hospital treatment for malformation The largest subgroups were cardiovascular (5 cases=1 4‰)

ble 2 *Structural malformations detected in the seven year study period*

Years	0–1	?	3	4	5	6	7	Total
:rocephalus	1							1
galocornea	1							1
tara-ta congenita					1			1
omalia papillae						1		1
omalia palpebrae	1							1
cta colli congenita		1					1	2
:fectus septi ventr	1							1
:fectus septi atrior							1	1
ıctus art persistens	?			1				3
latoschisis submuc			1					1
orbus Hirschsprung				1				1
plasia testis unilat							1	1
ypospadia	?			1			1	4
pispadia		1						1
yndactylia pedis	1							1
ysostosis cleidocran					1			1
nomalia dig man					1			1
nomalia pollicis							1	1
nomalia costae 1							1	1
inus pilonidalis					1			1
Mongolismus					1	1		2
Surdomutitas cong		2					1	3
	9	4	1	3	5	2	7	31

genito urinary (6=1 7%) and eye malformations (5 cases=1 4%) Separate from these children with structural malformations were 80 children with minor or debated defects (Table 3) All 80 had been treated in the hospital for their abnormalities The largest sub group was that of abdominal hernias which were found in 40 children (=11 3%)

These cases of debated or minor defects when added to those with structural malformations make a total of 111 children to form the group of all congenital defects

This leaves 137 children to form the all disorders group (Table 4) The most common abnormalities in this group were strabismus and refractive disorders (54 cases=

Table 3 *Congenital defects detected in the seven year study period*

	No	%
Hernia inguinalis	33	9 3
Hernia umbilicalis	3	
Hernia scrotalis	4	
Dislocatio coxae cong	1	3 4
Pectus excavatum		
Stenosis pylori	10	? 8
Retentio testis	9	? 5
Hydrocele	5	
Hydronephrosis	1	
Stenosis urethrae	1	
	80	6

Table 4 *Other disorders or abnormalities with prenatal etiology detected in the seven year study period*

	No	/
Vitia refractionis strabismus	54	15 3
Retardatio mentalis convulsiones	34	9 6
Phimosis	4	6 8
Stenosis canalis lacrim	11	3 1
Epicanthus	6	
Dyslalia	4	
Stridor laryngis cong	1	
Insuff cardiae	1	
Hyperplasia thymi	1	
Retardatio mentalis retinus pigmentosa strabismus	1	
	137	38 7

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(1) recorded in a cohort of 6789 children followed up for 13–14 years a frequency of congenital malformations of 2 9% Of these malformations 45 5% were diagnosed at the maternity hospitals and 54 5% subsequently The results in Mellin s (7) prospective series with 5531 pregnancies and a follow up period of 12 months show a similar increase in detection rate from 3 5 to 6 9% In McDonald s (6) series the high frequency of 16% is due to the fact that he included as malformations children with convulsions and brain injuries pyloric stenosis hernias strabismus refractive errors and cases of asthma and eczaema The definition of congenital malformations in McDonald s series corresponds to the widest definition of congenital malformations with all disorders or abnormalities with apparent prenatal etiology used in this study The possibility of over diagnosis has been discussed several times in the literature especially in relation to reports on congenital dislocation of the hip and malposition of the feet In the present study though erroneous diagnoses during the neonatal period were found the effect of this overdiagnosis on the frequency of congenital defects seems to be slight

The comparison of different epidemiological studies is a most difficult problem compounded by inconsistencies and arbitrariness both in the classification of malformations and in matching diagnoses to the predetermined categories Differences in interpretation therefore can hardly be avoided completely but this study shows that they can certainly be minimized by use of a classification system for malformations which would best be based on international agreement and include age adjusted incidence figures

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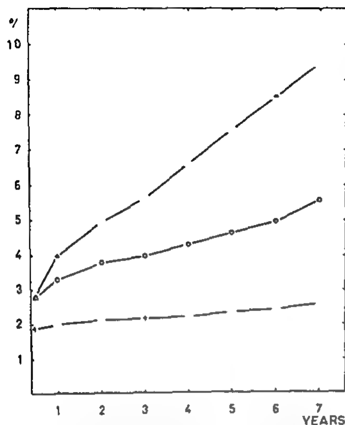


Fig 1 The cumulative detection rates of structural malformations all congenital defects and all disorders or abnormalities with prenatal etiology Δ—Δ All disorders or abnormalities with prenatal etiology O—O all congenital defects +—+ structural malformations

15 3‰) convulsion and mental retardation (34 cases=9 6‰) and phimosis (24 cases=6 8‰) Hospital examination was required in 93 cases

Fig 1 shows the cumulative detection rate as a function of time The final frequencies were 9 4% in the group of all disorders or abnormalities with prenatal etiology 5 6% in the group of all congenital defects and 2 6% in the structural malformation group

DISCUSSION

In most epidemiological studies on congenital malformations the malformations are recorded and the diagnosis made at birth or shortly after A follow up study both enables the natural history of the neonatally diagnosed abnormalities to be defined and the possibility for accurate observations concerning those congenital anomalies which are not undetected in the neonatal period (2 4 8)

The basic requirement for a successful follow up study is a sufficiently complete follow up so that the analysis can be made on unbiased proportion of the original material The rather high follow up rate (82 7%) in the present investigation was mainly due to the manageable size and defined geographical localization of the cohort and also to the well organized medical and health care in the chosen area Another advantage of a relatively small cohort and limited study is that all the data can be collected and analysed by one person on an individual basis An obvious drawback however in such a small cohort is that some subgroups are represented by numbers too small for meaningful statistical analyses On the other hand in the light of the literature it is apparent that in such follow up studies the size of the cohort is inversely proportional to the number of the defects observed (2 9)

The influence of the definition of the term congenital malformation on their frequency of occurrence is widely known (4) The present study indicates a trebling of their frequency if the definition is made wider than strictly structural malformations to include minor deviations and functional disturbances An increase in the cumulative detection rate as a function of time is also demonstrated

Two other follow up studies have been published from Finland and are comparable as far as definitions are concerned Hakosalo (3) followed a cohort of 6 147 children over a period of 10 years The congenital defects were treated as three categories i e congenital defects with 900 children congenital malformations (467 of these 900) and generally accepted malformations (203 of the 467) Perinatally the recorded frequencies were 9 8% 3 3% and 2 0% respectively Subsequently the figures rose to 14 6% 7 6% and 3 3% at the end of the study Amnell

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INCREASED INTRACRANIAL PRESSURE IN CYSTIC FIBROSIS

DANIEL KATZNELSON

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ABSTRACT Katznelson D (Department of Pediatrics A and the Harry Shwachman Clinic Sheba Medical Center Tel Hashomer Tel Aviv University Sackler School of Medicine Israel) Increased intracranial pressure in cystic fibrosis Acta Paediatr Scand 67 607 1978 —Three cystic fibrosis infants with severe respiratory distress had increased intracranial pressure (with bulging fontanels) which cleared parri passu with improvement in the chest condition It is proposed that the intracranial hypertension is a result of raised venous pressure itself secondary to the intrathoracic obstruction to venous return consequent on the bronchial obstructive disease

KEY WORDS Cystic fibrosis increased intracranial pressure

Transitory benign intracranial hypertension or pseudotumor cerebri in children has been ascribed to various causes such as otitis media (4) tetracycline (3) and vitamin A (7) That cystic fibrosis (CF) can be a cause is not widely nor adequately known and probably deserves emphasis since CF frequently takes the form of respiratory disease or diarrhea conditions for which antibiotics are often given and these may then wrongly be held responsible for the increased intracranial pressure

CASE PRESENTATIONS

Case I

[illegible] A male weighed 2950 g at birth progress was good He was admitted at the age of [illegible] months because of vomiting and cough

On admission weight was [illegible] 0 kg temperature normal breathing rapid 48/min pulse 112/min The chest was resonant air entry was markedly diminished no rales were heard fontanel was bulging.

X ray of chest showed hyperinflated lungs with low diaphragms Arterial gases (room air) were P_{aO_2} 9 3 kPa (70 mmHg) and P_{aCO_2} 4 7 kPa (35 mmHg) Sweat test was positive (quantity 0 2345 g Na 81 mmol/l Cl 95 mmol/l) Lumbar puncture revealed clear fluid at a pressure of 1 [illegible] mm water (in our experience normal range in this age is 80–90 mm H_2O) chemistry and bacteriology were negative Laryngeal swabs grew Pseudomonas aeruginosa and Klebsiella

The infant was started on chloramphenicol and co-trimoxazole prednisone 5 mg thrice daily physiotherapy and oxygen His condition was stable with the respiratory rate at times rising to over 100/min Antibiotics were changed to intravenous gentamycin with carbenicillin continued for another two weeks and then changed to cephalothin i m There was steady improvement Respiration improved greatly arterial P_{aO_2} was 12 6 kPa The fontanel no longer bulged and two weeks later he was discharged home

Case II

E H male birth weight 3330 [illegible] was operated on at the age of 5 days because of meconium ileus CF was confirmed by sweat test (Na 80 mmol/l Cl 100 mmol/l) At the age of 1 month he was transferred to our service Weight was 3700 g temperature normal respiratory rate 60–80 min pulse 140/min Suprasternal retraction was observed air entry was diminished symmetrically no adventitious sounds were heard heart normal X ray of chest showed clear lung fields with low diaphragms and small heart Blood count urea glucose electrolytes proteins and urine were within normal limits Arterial gases (breathing room air) P_{aO_2} 10 3 P_{aCO_2} 3 1 kPa pilocarpine iontophoresis sweat test was positive (0 7778 g Na 88 mmol/l Cl 96 mmol/l) He was given cephalothin fluids and 75 mg hydrocortisone i v daily for 4 days and elemental diet (vivonex) orally The weight steadily improved at a rate of about 200 g per week On the third day mild edema appeared in the eyelids and legs (after a weight gain of 170 g) blood proteins were 52 g/l albumin 32 g/l globulin 20 g/l The fontanel was bulging air entry was greatly decreased to both lungs with marked wheezing Fundus was normal lumbar puncture within normal limits pressure not measured Cephalexin was given by mouth as was triamcinolone [illegible] mg/day The child's condition improved bowel motions were now per rectum Weight gain was steady and the fontanel was flat On the

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9th day the fontanel bulged again lumbar puncture was normal except for a pressure of 135 mm H_2O X ray of chest again showed clear lung fields with signs of trapped air After another week the mild edema of eyelids and legs cleared and the fontanel was flat On the 36th day he was discharged home on cephalexin and triamcinolone 4 mg/day weighing 4 0 kg

Case III

D G female birth weight was 3 4 kg The infant had frequent bowel motions vomiting and no weight gain At the age of 3½ months CF was diagnosed elsewhere She was admitted to our service after 3 days of fever severe dyspnea and cough Weight was 4 0 kg temperature 37 6 C breathing 85–100/min marked suprasternal and intercostal retractions the chest was hyper resonant with severely diminished air entry heart rate 150/min regular no murmurs fontanel bulging fundus normal Lumbar puncture was normal except for a pressure of 260 mm H_2O X ray of chest showed hyperaeration with low diaphragms Blood count and electrolytes and urine were within normal limits Arterial gas (room air) P_{O_2} 6 1 P_{CO_2} 6 4 kPa Pilocarpine iontophoresis sweat test gave positive results (quantity 0 3713 g Na 106 mmol/l Cl 116 mmol/l) The infant was given oxygen cephalothin i v and physiotherapy

Two days later there was improvement breathing 50–70/min and the fontanel no longer bulged On the 5th hospital day dyspnea recurred breathing was 90–100/min pulse 160/min and the fontanel again bulged Breath sounds were diminished X ray showed hyperinflation Cephalothin was changed to gentamycin and carbenicillin i v The child improved and gained weight The fontanel was flat On the 16th hospital day and after 2 days of fever (up to 39 C) there was an increase in the cough the fontanel was again full and bulging X ray showed clear lung fields with signs of hyperinflation Improvement again followed the intravenous antibiotics were stopped and chloramphenicol was given orally The infant continued to improve the fontanel was flat breathing improved and after 8 weeks in hospital she was discharged

DISCUSSION

The three CF infants reported had each experienced several weeks of increased intracranial pressure as evidenced by a bulging fontanel and raised pressure on lumbar puncture Intraspinal pressure in benign intracranial hypertension may fluctuate from periods of normal pressure to shorter periods of elevated pressure (5) All presented with severe obstructive bronchopathy which is the underlying basic pulmonary disturbance in CF (8) The latter understandably led to pulmonary air trapping and increased intrathoracic pressure interference in venous return which in turn caused a rise in venous pressure It is reasonable to accept the latter as the mechanism responsible for both the increased intra cranial pressure as demonstrated experimentally (5) as well as for the edema Tetracyclines and vitamin A known to cause bulging of the fontanel were not given to these infants and so cannot be implicated Steroids were given to cases 1 and 2 and although there may be a causal association between steroids and increased intracranial pressure (2) it is unlikely that they were responsible in this series Primarily this is because in case 1 the bulging fontanel was already present at the time of admission and before steroids were given and secondly case 3 did not receive steroids Also in case 2 the increased pressure as manifested by a bulging fontanel waxed and waned synchronously to changes in the degree of respiratory embarrassment unrelated to the steroid administration and presumably related to the degree of intrathoracic pressure thus further suggesting a direct causal relationship between the two Moreover increased intracranial pressure due to steroids usually develops at the time of decreasing or termination of the treatment (2) and not as in case 2 here at the start of treatment

CF has been extensively studied for decades and there are many excellent descriptions of the clinical picture (6 8 9) However increased intracranial pressure or bulging fontanel have not been mentioned except in a single report of a bulging fontanel that appeared after therapy with consequent nutritional improvement and was ascribed to rapid brain growth as a catch up effect (1) It is probably important to know that severe respiratory distress in CF may be a cause of increased intracranial pressure in order to allay fears in the choice of drugs For the withholding of antibiotics and steroids on the unjustified suspicion that they may be the cause of the increased intracranial pressure may by depriving the child of valuable drugs delay or even prevent the latter s cure

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RENAL FUNCTIONAL ADAPTATION IN THE REMNANT KIDNEY IN PATIENTS WITH RENAL AGENESIS AND IN PATIENTS NEPHRECTOMIZED IN CHILDHOOD

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ABSTRACT Aperia A Broberger O and Wilton P (Department of Paediatrics Karolinska Institute St Goran s Children s Hospital Stockholm Sweden) Renal functional adaptation in the remnant kidney in patients with renal agenesis and in patients nephrectomized in childhood Acta Paediatr Scand 67 611 1978.—The renal response to volume expansion was determined in four patients with renal hypertrophy due to unilateral renal agenesis (URA) and in four patients with renal hypertrophy due to nephrectomy (Nz) Four healthy controls were also studied The studies were performed during water diuresis and following i v infusion of isotonic saline solution Conventional clearance techniques were used GFR and PAH clearence were increased to about the same extent in Nz and in URA Fractional Na excretion was highest in the Nz group and lowest in the control group It was higher in the Nz group than in the URA group Fractional water excretion (V/GFR) and free water clearance (C_{H_2O}) were also determined and the results indicate that the high fractional excretion of Na from the hypertrophied kidney can be attributed to reduced fractional reabsorption of filtrated Na both in the proximal and the distal tubules The fractional Na reabsorption in the distal tubule appears to be higher in URA than in Nz It is concluded that glomerular tubular balance for Na is more similar to that found in healthy controls if the stimulus of hypertrophy occurs prenatally than if it occurs postnatally

KEY WORDS Nephrectomy renal agenesis renal hypertrophy renal function sodium excretion

Compensatory renal hypertrophy is characterized functionally by increased glomerular filtration rate (GFR) This is well documented in both patients and experimental animals It is still uncertain however whether glomerular and tubular function increase to the same extent in the hypertrophied kidney This question is of clinical importance since a change in glomerular tubular balance will influence the homeostatic efficiency of the kidney

In the experimental animal with only one kidney the proportion of filtered Na^+ that is reabsorbed is generally reported to be low This results in an increased fractional excretion of Na The increase is so pronounced that the absolute Na^+ excretion has been reported to be higher in animals with one than in those with both kidneys (21)

The capacity of the hypertrophied human kidney to excrete sodium has not been systematically determined This study was therefore designed to compare the natriuretic response to volume expansion in patients with only one kidney and in healthy controls The structural characteristics of renal hypertrophy in the experimental animal vary with the developmental stage of kidney when contralateral nephrectomy is carried out (6) For this reason nephrectomized patients and renal agenesis patients were included

MATERIAL

Three groups of subjects were studied One group consisted of four patients nephrectomized in childhood because of unilateral kidney disease (Nz)

The second group consisted of four patients with unilateral renal agenesis (URA) The third group consisted of four healthy adult controls without a history of kidney

Table 1 *Clinical data of patients studied*
UTI=urinary tract infection

Patient	Sex	Age at nephrectomy (y)	Symptoms	Kidney disease	Age at the time of study (y)
A Nephrectomized					
MB	M	0 25	Failure to thrive UTI	Congenital hydro nephrosis dysplasia	17
Po	M	2	Hematuria abdominal tumor recurrent UTI	Hydronephrosis	10
LM	F	6	Traumatic rupture	Normal kidney	13
AA	F	11	Recurrent UTI	Segmental dysplasia	20
B Agenesis					
LB	M	–	Abdominal trauma	–	12
BP	M	–	Enuresis	–	19
IA	F	–	Recurrent UTI	–	14
AJ	F	–	Recurrent UTI	–	16

disease (C) Pertinent clinical data on the patients are presented in Table 1

All of the patients were in good general health and none had symptoms of urinary tract infection during the year prior to the study They were normotensive They were kept on a normal salt diet for a minimum of three days before the study Urograms were obtained in all but two subjects both with URA Renal hypertrophy was present in the Nz and URA patients but no other ab normalities of the kidney and urinary tract were detected Children were hospitalized for one week whereas adults were out patients Informed consent was obtained from all subjects Permission to carry out this study was ob tained from the Ethical Committee of the Karolinska Institute

METHODS

The subjects were allowed to drink water but not ingest food for 12 hours before the study Sustained water diuresis was induced with an oral intake of tap water of 20 ml/kg BW one hour before the study The glomerular filtration rate (GFR) and renal plasma flow (C_{PAH}) were determined by the clearence of inulin and PAH using a continuous infusion containing 0 001 g inulin/min/kg BW and 0 0002 g PAH/min/kg BW after a prime dose of 0 06 g inulin/kg BW and 0 009 PAH/kg BW Urine was collected through an indwelling catheter during 10–15 min periods The high urinary flow made such short collecting periods possible Blood was collected in the middle of each period from an indwelling catheter in a peripheral vein

When a steady flow was obtained with an osmolality of less than 100 mosmol/kg one or two urine samples were collected An isotonic saline solution containing 147 mmol Na^+ 4 mmol K^+ 4 6 mmol Ca^{++} and 155 6 mmol Cl per 1 000 ml was thereafter infused intravenously at a rate of 10–17 ml/min so that a volume equivalent to 3% of BW was infused during 45 min The study was then ter minated

Analysis

Analyses of inulin in serum and urine were made with the anthron method (12) PAH was analysed using the method of Smith et al (20) Sodium in serum and urine was deter mined with a flame photometer and osmolality was meas ured cryoscopically with a Knauer microosmometer

Abbreviations and calculations

Clearence of inulin (C_I) clearence of paraaminohippuric acid (C_{PAH}) clearence of sodium (C_{Na}) and osmolar clear ence (C_{osm}) were obtained by standard calculations Free water clearence (C_{H_2O}) was calculated with the formula $C_{H_2O}=V-C_{osm}$ where V represents the urine flow Filtra tion fraction stands for C_I/C_{PAH} Fractional excretion of sodium (FE_{Na}) was calculated with the formula $U_{Na}\times V\times 100/C_I\times P_{Na}$ where U_{Na} and P_{Na} represents the sodium concentration in urine and plasma respectively

One of the purposes of this study was to determine the proportional change in tubular and glomerular functions particularly with regard to the handling of water and sodium This was assessed by relating all tubular para meters to the GFR and then comparing the different groups For statistical analyses Student s t test was used

RESULTS

The glomerular filtration rate (GFR) in the C group averaged 103 ml/1 73 m²/min (Table 2) Since there is no constant difference between the function of the two kidneys in healthy in dividuals (13) it can be assumed that the GFR in one kidney should average 51 5 ml/1 73 m²/min under these experimental conditions The GFR in the Nz group averaged 81 ml/1 73 m²/min and is therefore significantly increased ($p<0.05$) The GFR in the URA group aver

Table 2 *Summary of data during isotonic volume expansion*

	GFR ml/1 73 m /min		C /C		FE		V/GFR		C_{H_2O}/C +C_{H_2O}	
	C	VE	C	VE	C	VE	C	VE	C	VE
Volunteers n=4										
Mean	103	96	0 18	0 [illegible]0	1 33	3 58	0 13	[illegible] 15	0 87	0 76
S E M	4	5	0 01	0 01	0 1[illegible]	0 4[illegible]	0 01	0 01	0 0[illegible]	0 03
Nephrectomized n=4										
Mean	81	91	0 22	0 23	[illegible]	6 78	0 14	[illegible] 18	0 8[illegible]	0 59
S E M	8	5	0 03	0 02	0 30	1 35	0 01	0 01	0 02	0 05
Renal agenesis n=4										
Mean	89	89	0 20	0 21	1 60	4 64	0 15	0 17	0 85	0 72
S E M	[illegible]	2	0 01	0 01	0 30	0 84	0 01	0 01	0 03	0 03

C=Control periods VE=after volume expansion

aged 89 ml/1 73 m²/min and is also significantly increased in comparison to the controls ($p <$ 0 02) Although the GFR in the remaining kidney in the URA patients was higher than that in the remaining kidney in the Nz patients the difference was not significant The C_{PAH} showed the same trend as the GFR This resulted in a fairly constant filtration fraction in all patients No significant change in GFR or C_{PAH} was induced by saline infusion in any group

Patients with compensatory renal hypertrophy showed a change in the renal handling of sodium Fractional excretion of sodium (FE_{Na}) was consistently higher in patients with one kidney than in controls and highest in patients in the Nz group The differences in sodium excretion became more pronounced during isotonic volume expansion (Fig 1)

During water diuresis a negligible amount of fluid is reabsorbed distal to the proximal tubule Thus the ratio of urine flow to GFR can be used as an index of the volume of fluid delivered to the distal tubule (4) During saline infusion V/GFR increased in all patients studied (Fig 2)

The average increase was somewhat greater in the Nz and URA groups than in the C group but the difference between individuals with one and those with two kidneys was insignificant

During water diuresis free water will be excreted in proportion to selective Na⁺ reabsorption in the water impermeable distal tubule (8) The amount of filtered sodium that is not reabsorbed will be excreted in the urine (C_{Na}) Thus the sum of free water clearence and Na⁺ clearence will be an index of the total amount of sodium delivered to the distal tubule (14) Consequently the quotient of C_{H_2O} to $C_{Na}+C_{H_2O}$ will represent the fractional distal tubular sodium reabsorption During control conditions the quotient ($C_{H_2O}/C_{Na}+C_{H_2O}$) was similar in all groups but during saline infusion it decreased significantly in all groups ($p <$ 0 01) The decrease was more pronounced in the Nz group than in the C and the URA

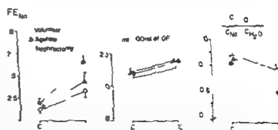


Fig 1 Fractional sodium excretion before (*C*) and after isotonic saline infusion equal to 3% body weight (*VE*) Mean ± S E M in volunteers and in nephrectomized and agenesis patients

Fig 2 Fractional water excretion before (*C*) and after isotonic saline infusion equal to 3% bodyweight (*VE*) Mean ± S E M Symbols as in Fig 1

Fig 3 Fractional distal tubular Na reabsorption before (*C*) and after isotonic saline infusion equal to 3% body weight (*VE*) Symbols as in Fig 1

groups (Fig 3) Thus during saline infusion $C_{H_2O}/C_{H_2O}+C_{Na}$ was significantly lower in the Nz group than in the C ($p<0.05$) and the URA groups ($p<0.05$)

DISCUSSION

The fraction of filtered sodium excreted in the urine is increased in patients with only one kidney Thus tubular reabsorption of Na^+ is not increased in proportion to GFR in compensatory renal hypertrophy The discrepancy between the capacity to filter Na^+ and the capacity to reabsorb Na^+ is larger in the Nz patients than in the URA patients The relatively high fractional Na^+ excretion in compensatory renal hypertrophy appears to be an adaptation that protects the subject from Na^+ retention

By increasing the fractional Na^+ excretion the kidney with compensatory renal hypertrophy can excrete at least the same amount of sodium per unit of body surface as two normal kidneys In fact the capacity to excrete sodium appears to be overcompensated in nephrectomized patients The finding of a high Na^+ excretion in patients with only one kidney accords with the natriuresis observed in the rat and dog after reduction of renal mass (7 9 11 15 18) In experimental studies micropuncture technique has usually been used These studies of function in different parts of the nephron have suggested that proximal tubular Na^+ reabsorption is reduced relative to the filtered load (1 7 10) and that the distal tubular Na^+ reabsorption is if anything enhanced although not sufficiently to prevent sodium diuresis (7) Clearence studies are a crude means of localizing changes in sodium reabsorption within the nephron but it is generally agreed that they give some information on the amount of fluid delivered to the distal tubule and the amount of sodium reabsorbed in the distal tubule provided they are performed during water diuresis (5) The amount of fluid delivered to the distal tubule is somewhat higher in Nz and URA patients than in controls This indicates that the fraction of filtered sodium that is reabsorbed in the proximal tubule is reduced in Nz and URA patients The results also suggest that the fraction of filtered sodium that is reabsorbed in the distal tubule is reduced in Nz patients but not in URA patients A relative decrease in distal tubular sodium reabsorption in Nz patients is contrary to the findings in experimental animals (7 16) This inconsistency may be due to species differences There are however, several differences between reported experimental studies and the present study

One important variable is the difference in observation time In most experimental studies in rats the time interval between nephrectomy and functional studies does not usually exceed three weeks Even if one takes into account the short life span of the animals used this observation time is much shorter than in the present study

URA patients differ somewhat from Nz patients both with regard to glomerular and tubular functions The GFR in Nz patients is in the same range as in kidney donors studied one month to four years after nephrectomy (3 17) The average GFR in URA patients is higher than the average GFR in Nz patients The difference between the groups is not significant in this study but in a larger material consisting of 10 URA children and 8 Nz children Seipelt et al (19) found a significantly higher GFR in URA children Although the GFR was higher in URA than in Nz children the urinary Na excretion was lower in URA children It can therefore be concluded that the proportion of tubular Na reabsorption to GFR increases more in URA patients than in Nz patients

In URA patients the stimulant to renal hypertrophy may exist prenatally Three of the four Nz patients had unilateral renal disease that existed prenatally It has been shown however that even in advanced unilateral renal disease the compensatory increase in renal mass will be much more pronounced after than before nephrectomy (2) It thus seems likely that the differences in renal func

tion observed in URA and Nz patients can be attributed to the stage of renal development at which the maximal stimulus to renal hypertrophy occurred

This is in line with results from previous structural studies which have shown that if compensatory renal hypertrophy is initiated during early development of the kidney structural growth will be more differentiated (6) The fact that glomerular tubular balance with regard to sodium is better maintained in URA than in Nz patients does not necessarily mean that prenatal adaptation results in a more optimal homeostatic function than postnatal adaptation The hazards of sodium retention in URA patients may have more serious consequences than the risks of sodium losses in Nz patients

ACKNOWLEDGEMENTS

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RELIABLE DIAGNOSIS OF THE MAJOR TYPE OF CYSTIC FIBROSIS WITH FIBROBLAST CULTURES

A Double Blind Study

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ABSTRACT Hosli P Kollberg H and Vogt E (Department of Molecular Biology Institut Pasteur Paris France and the Department of Paediatrics University Hospital Umeå Sweden) Reliable diagnosis of the major type of cystic fibrosis with fibroblast cultures A double blind study Acta Paediatr Scand 67 617 1978 —A double blind study has been carried out to demonstrate that the most common type of cystic fibrosis (CF) can be reliably diagnosed with skin derived fibroblast cultures Alkaline phosphatase (AlP) activity has been measured in 6 normal controls 12 CF heterozygotes and 6 CF homozygotes before and after stimulation with Tamm Horsfall-glycoprotein (THP) isoproterenol and theophylline (THP induction test) The mean AlP activities after THP induction were 8 8 12 7 and 34 6 for the three different genotypes respectively There was no overlap between the values of CF homozygotes on the one hand and the values of CF heterozygotes and normal controls on the other hand All 24 specimens were correctly diagnosed in the present double blind study indicating the very high degree of reliability of the THP induction test in the detection of the predominant type of cystic fibrosis with fibroblast cultures Normal controls and CF heterozygotes could not be discriminated on an individual basis but as a group the CF heterozygotes displayed higher AlP values

KEY WORDS Alkaline phosphatase cystic fibrosis fibroblast cell cultures Tamm Horsfall glycoprotein ultramicrotechniques

Although cystic fibrosis (CF) is the most common lethal to semi lethal genetic disease of children and young adults in Caucasian populations its underlying biochemical defect has so far been elusive Earlier observations pointed to a possible abnormality in lysosomes (1 2 17 18 19) More recently we have shown that in the majority of CF fibroblast cultures lysosomal hydrolases acid and alkaline phosphatase (AlP) can be induced with Tamm Horsfall glycoprotein (THP) (8) and that in these cultures many lysosomal enzymes leak out of the cells into the extracellular space i e the culture medium (11) These observations indicate that at least the predominant type of cystic fibrosis qualifies as a hereditary disease of the intracellular digestive tract (12) and they point towards the primary defect in cystic fibrosis it is likely that comparable to I-cell disease (16) there is a defect in the recognition site of lysosomal enzymes (5) Previously it has been shown that the AlP induction of fibroblast cultures with Tamm Horsfall glycoprotein discriminates between CF homozygotes on the one hand CF carriers and normals on the other hand and it has been suggested that the THP induction test might serve as a practical tool for the diagnosis—particularly the prenatal diagnosis—of the common type of cystic fibrosis (8) The purpose of the present double blind study is to demonstrate the high degree of reliability of the THP induction test when used with skin derived fibroblast cultures

MATERIALS AND METHODS

In Umeå (Sweden) 24 skin biopsies were taken from 6 normal controls (surgical patients) aged 1 to 51 years I

and a definite effect on CF homozygotes The AIP activities of THP induced fibroblasts show in contrast to non induced cells no overlap between CF homozygotes on the one hand CF carriers and normal controls on the other hand ($p<0.001$ comparing the means) From one sided tolerance limits (the variable was assumed to display an approximately normal distribution) it was concluded that 99% *of THP induced CF homozygotes have AIP* activities higher than 19×10^{-13} moles methyl umbelliferone liberated per 20 cells per hour and that more than 95% of CF carriers and more than 97 5% of normal controls have AIP activities lower than 19×10^{-13} moles methyl umbelliferone liberated per 20 cells per hour It is not possible to discriminate with individual cell cultures between induced controls and induced CF carriers however as a group the THP induced CF heterozygotes display clearly higher AIP activities than the THP *induced* normal controls ($p<0.01$ comparing the means)

DISCUSSION

The importance of employing ultramicrotechniques (*6 7 9 10*) must be stressed First they allow AIP activities to be expressed per cell instead of per total cell protein this is essential since it is known that the protein content of CF fibroblasts is abnormal (3) which implies that the expression of *enzyme* activities per total cell protein could be very misleading

Second ultramicrotechniques may eventually make rapid prenatal diagnosis of cystic fibrosis possible in early pregnancy with very small amounts of cell material (7)

The double blind test was designed to mimic the conditions which will prevail in prenatal diagnosis in families at risk for cystic fibrosis where the chance of encountering a CF homozygote is 0 25 a CF-carrier 0 5 and a *normal* 0 25 All CF homozygotes on the one hand normal controls and CF carriers on the other hand have been clearly discriminated The probability of correctly predicting the coded sequence of the 24 specimens on an accidental basis is as low as 0 000 007 The present double blind study seems therefore to demonstrate the reliability of the THP induction test (if used with skin derived fibroblast cultures) in the diagnosis of the predominant type of cystic fibrosis Until now we have analysed 59 skin derived fibroblast cell strains from CF patients (13) With the exception of two cell strains derived from one sibship with cystic fibrosis all cell strains were THP induceable and (as far as tested) leaky for lysosomal enzymes (11) At the present time it is unknown if these rare exceptions are due to an entirely different biochemical defect or to methodological errors

Table 1 shows that the THP induced CF heterozygous fibroblasts have higher mean AIP activities than the THP induced normal control cells This increase of AIP activity is however so small that it can not discriminate on an individual basis between CF heterozygotes and controls Recently we have demonstrated that the basic defect of cystic fibrosis leads to a multiple leakage of lysosomal enzymes into the extracellular space in fibroblast cultures from both CF homozygotes and CF heterozygotes (11) These two observations indicate that it may be possible to develop a test which discriminates—with individual cell cultures—between the three genotypes i e CF homozygotes CF heterozygotes and normal persons

It must be underlined that the present study indicates the reliability of the THP induction test only for skin derived fibroblast cultures Amniotic fibroblast cultures and amniotic intermediary cell type cultures seem to behave in a way comparable to skin derived fibroblast cultures (13) Preliminary studies have on the other hand shown that the base line activity of alkaline phosphatase is higher in epithelial amniotic cells than in fibroblast like cells (14) and the reaction of these cells on THP induction will have to be studied in detail A careful analysis *of the* behaviour of the different

Table 1 *Alkaline phosphatase activities in fibroblast cultures from normal controls CF heterozygotes and CF homozygotes with and without Tamm Horsfall glycoprotein induction*

AlP activity is expressed in 10^{-13} moles of substrate converted/20 cells/hour. Each value represents the mean of five independent determinations. The mean coefficient of variation (including analytical and biological variations) of the five determinations is 15.9%. Δ Differential AlP activity of induced minus non induced cells

	Controls 3♂/3♀			Heterozygotes 7♂/5♀			Homozygotes 3♂/3♀		
	Non induced	Induced	Δ	Non induced	Induced	Δ	Non induced	Induced	Δ
Individual values	5.5	5.5	0.0	5.5	10.5	5.0	12.0	36.7	24.7
	8.0	5.0	−3.0	6.0	10.7	4.7	12.2	29.5	17.3
	9.2	12.0	2.8	6.5	9.0	2.5	14.0	28.7	14.7
	9.5	10.7	1.2	6.7	8.7	2.0	14.0	37.0	23.0
	10.5	9.0	−1.5	7.7	12.7	5.0	15.5	38.7	23.2
	11.0	10.2	−0.8	8.5	11.2	2.7	16.2	36.6	20.4
	–	–	–	8.7	11.5	2.8	–	–	–
	–	–	–	10.7	11.5	0.8	–	–	–
	–	–	–	11.2	18.0	6.8	–	–	–
	–	–	–	12.0	15.7	3.7	–	–	–
	–	–	–	12.2	18.2	6.0	–	–	–
	–	–	–	12.7	14.0	1.3	–	–	–
Mean values	9.0	8.7	−0.2	9.0	12.6	3.6	14.0	34.5	20.6
95% conf int	6.9–11.1	5.8–11.8	−1.9–2.3	7.3–10.7	10.6–14.7	2.4–4.8	12.2–15.8	30.1–39.1	16.5–24.7
Range	5.5–11.0	5.0–12.0	−3.0–2.8	5.5–12.7	8.7–18.2	0.8–6.8	12.0–16.2	28.7–38.7	14.7–24.7

obligate CF heterozygotes (parents of CF children) aged 29 to 53 years and 6 CF homozygotes aged 2 to 24 years who displayed typical clinical symptoms and elevated sweat electrolyte concentrations (Gibson Cooke method (4) double determinations). The patients and the controls were not matched with reference to age nor sex. We did not regard this as necessary as we have so far not found any change with age among our heterozygotes and homozygotes and no sex differences (14). From the skin biopsies which were all handled in the same way fibroblast cultures were established with conventional tissue culture techniques in Eagle's medium MEM supplemented with 10% fetal calf serum. The 24 vigorously growing cultures were coded in Sweden in a way to completely mix the sequence of the three genotypes and then transferred by air mail to France.

In Paris the cultures were switched to HAM F10 medium (containing 15% fetal calf serum) and used between the 7th and 14th subculture for the THP induction test. The induction protocol was slightly modified as compared to the one described previously (8, 10, 11, 13). The fibroblasts were seeded into *Plastic Film Dishes (PFD's)* (6, 9) (about 8 000 cells per cm² culture surface) and grown for 80 hours. Eight hours after seeding the AlP induction was started by adding 100 μg Tamm Horsfall glycoprotein per ml of medium. 24 hours later isoproterenol (final concentration 1×10^{-6} M) plus theophylline (final concentration 1×10^{-3} M) and again 24 hours later isoproterenol (final concentration 1×10^{-6} M) were added to the same THP induction medium. The non induced cell cultures were run simultaneously but with medium without inducers. Then the cells were washed three times with balanced salt solution shock frozen in liquid nitrogen and lyophilized in conventional ways (10). All enzyme assays were carried out with previously described ultramicrotechniques (6, 9, 10). Plastic film leaflets carrying 20 lyophilized fibroblasts were dissected from the plastic film bottom of *PFD's* in an air conditioned room 18°C relative humidity 30–40% making use of a stereomicroscope. The plastic film leaflets with the adhering cells were transferred into *Parafilm Micro Cuvettes (PMC's)* (10) containing 0.3 μl of the following substrate: 4 methylumbelliferyl phosphate (Koch and Light G.B.) 5.5 mmoles/liter in AMP buffer (200 mmoles/liter pH 9.6) with 0.05% BSA. The sealed *PMC's* were incubated for 2 hours at 37 C in a water bath, subsequently the enzyme reaction was stopped by diluting the 0.3 μl of reaction mixture with 500 μl of carbonate buffer (500 mmoles/liter pH 10.7). The amount of liberated methylumbelliferone was determined with a Perkin Elmer MPF-4 spectrophotofluorimeter (excitation wave length 360 nm, emission wave length 448 nm) (10). The statistical procedures used to determine the diagnostic reliability have been described earlier (15).

RESULTS

The Swedish code was not disclosed before the 24 cell strains had been tested twice in Paris and definitely diagnosed. Table 1 demonstrates that in the 24 fibroblast cultures tested the THP induction has no effect on normal controls, a slight effect on CF heterozygotes

Acta Pædiatr Scand 67 621–631 1978

RELATIONSHIP OF MATERNAL SMOKING TO MORBIDITY AND MORTALITY OF THE CHILD UP TO THE AGE OF FIVE

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ABSTRACT Rantakallio P (Department of Paediatrics and Department of Public Health University of Oulu Oulu Finland) Relationship of maternal smoking [redacted] morbidity and mortality of the child up to the age of five Acta Paediatr Scand 67 621 1978.—The effect of maternal smoking during pregnancy on the morbidity and mortality of the child up to the age of five was studied in 12068 births The children of the smokers were compared with those of controls of similar age parity marital status and place of residence Perinatal mortality was no higher among the smokers but postneonatal mortality from 28 days to 5 years was almost significantly ($p<0.05$) higher The children of the smokers were highly significantly ($p<0.001$) more often hospitalized in pediatric departments the difference being clearest below the age of one The average duration of hospital admissions was longer among the children of the smokers and similarly the numbers of visits to the doctor and hospital admissions to any hospital under the age of one were more frequent among the children of the smokers Respiratory diseases caused highly significantly more hospitalizations among these children

KEY WORDS Maternal smoking perinatal mortality childhood mortality hospital admission out patient visits respiratory diseases

The fact that maternal smoking during pregnancy lowers the birth weight of the offspring is well documented Studies on the relationship between maternal smoking and perinatal mortality are also numerous even though the findings still contain some contradictory points (1–4 8–12 15 17 19–21 25 26 31 32 37–40) Studies on the correlation between maternal smoking and morbidity and mortality during later childhood are relatively rare however Comstock & Lundin (8) reports that the childhood mortality rate up to the age of 11 was higher among children whose mothers smoked and impaired health in the children of smokers has been investigated in several studies (5 7 13 18)

The present study investigates the effect of maternal smoking on morbidity and mortality up to age of five in a series from Northern Finland in which maternal smoking habits were recorded during pregnancy

MATERIALS AND METHOD

The series consists of 12068 pregnant mothers from the two northernmost provinces of Finland Oulu and Lapland The investigation was started at the sixth or seventh month of pregnancy in the antenatal clinics and covered 96% of all deliveries in 1966 (26) Twin births numbered 163 and single births 11905 The many biological and socio-economic characteristics of the mother and family which were examined included the mother's smoking habits Each mother was asked whether she had been a regular smoker before pregnancy and if so how much she had smoked whether she had changed her habits during pregnancy and if so how

The mothers were divided into three categories in the following way

(*a*) *non smokers* those who never smoked or who had stopped smoking during the first two months of pregnancy

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In 554 mothers (4.6) smoking data was lacking or incomplete or in a few cases unclassifiable e g when the mother did not smoke at the beginning of pregnancy but started later The non smokers amounted to 9695 mothers 80.3% of the total and comprised 9176 who had never

amniotic cell types on THP induction will tell us if the THP induction test can be used for prenatal diagnosis of cystic fibrosis

ACKNOWLEDGEMENTS

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The present study investigates the effect of maternal smoking on morbidity and mortality up to age of five in [redacted] series from Northern Finland in which maternal smoking habits were recorded during pregnancy

MATERIALS AND METHOD

The series consists of 12 068 pregnant mothers from the two northernmost provinces of Finland Oulu and Lapland The investigation was started at the sixth or seventh month of pregnancy in the antenatal clinics and covered 96% of all deliveries in 1966 (26) Twin births numbered 163 and single births 11 905 The many biological and socio-economic characteristics of the mother and family which were examined included the mother s smoking habits Each mother was asked whether she had been a regular smoker before pregnancy and if so how much she had smoked whether she had changed her habits during pregnancy and if so how

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In 554 mothers (4 6%) smoking data was lacking or incomplete or in a few cases in lassifiable e g when the mother did not smoke at the beginning of pregnancy but started later *The non smokers* amounted to 9695 mothers 80 3% of the total and comprised 9 176 who had never

Table 1 *Perinatal mortality among the smokers and their controls*
Series is divided into social groups according to father s occupation[a]

Social group	Smokers: Still births	Smokers: Neonatal deaths first 28 days	Smokers: All births	Smokers: Perinatal mortality per 1000	Controls: Still births	Controls: Neonatal deaths first 28 days	Controls: All births	Controls: Perinatal mortality per 1000
I+II	4	5	320	28 1	4	5	402	22 4
III+IV	16	14	1 197	25 1	10	11	1 074	19 6
Farmers	1	3	157	25 5	4	4	205	39 0
Not known[b]	2	3	170	29 4	3	3	163	36 8
Total	23	25	1 844	26 0	21	23	1 844	23 9

[a] Based on the social standing of the occupation in question (36)
[b] Mostly mother unmarried

smoked and 519 who had stopped smoking during the first two months of pregnancy *The mothers who smoked* totalled 1819 1515 or 12 6% of the total number smoked less than 10 cigarettes per day while 304 (2 5%) smoked at least 10 cigarettes per day

The mean number of cigarettes smoked per day by the light smokers was 5 2 at the beginning of pregnancy and 3 9 in the middle of pregnancy The corresponding figures for the heavy smokers were 15 3 and 12 2 respectively

A control was chosen for each mother who smoked from among the non smokers so that the number of children born was the same the marital status was the same the age was the same within a range of ±2 years and the parity was the same if it was I and otherwise of the same order II or III IV or V and VI or over The place of residence was checked for similarity on three scores situated in the same province having the same level of public services taxable incomes of families and other development scores (24) all the 81 communes of the area being divided into four classes and being similar in population density (town–village–remote village) By this manoeuvre 1750 mothers out of the total of 1819 were assigned controls and 69 remained for whom it was impossible to find one For these the limits of dissimilarity were widened in one or two of the characteristics with exception of the number of children born marital status and parity I and VI+ in which the limits were kept as indicated

All hospitalizations of the study children in the pediatric departments of the four central hospitals in the area were recorded by the members of the study group in 1972 By the time the child concerned had reached the age of 4 7 6% of the families of the smokers and 7 0% those of controls had moved out of the study area No inquiries were made concerning hospitalizations outside study area

The analysis of the diagnoses given during hospitalization employs largely the main categories of diseases and these are grouped on the basis of the manual for the statistical classification of diseases and injuries (6) in official use in Finland since 1969 which was in turn compiled with reference to the recommendations of WHO The classification is identical with that in use in Sweden but differs in certain minor respects from the International Classification of Disease 1965 revision of WHO (14)

A questionnaire concerning the growth development and health of the children at the ages of 6 and 12 months was sent to the children welfare centres in the study area in 1967 This was returned by 85 3% of the smokers and 85 6% of the controls In this connection *all visits to the doctor and hospitalizations in departments other than pediatric departments in central hospitals and all admissions to local hospitals* supervised by a general practitioner were recorded

The data concerning *deaths* up to the age of 5 years were collected from the Population Registration Centre and the *causes of death* from the Central Statistical Office Since the great majority of deaths occurred before 1969 the earlier edition of the classification of diseases (22) was used in grouping the causes of death From the point of this series the greatest difference between the two editions lies in the causes of perinatal morbidity and mortality the former revision classifying most of the infectious diseases during the first 28 days into this group Thus under this system practically all causes of death others than accidents and congenital malformations during the first 28 days were classified into this category

Stillbirths were recorded at the postnatal clinics in connection with the other data (26) and as of 1966 all dead foetuses with a birth weight of 600 g or over were recorded as stillbirths in Finland

The follow up studies concerned the whole study group not only the smokers and their controls In testing significance the Student s t test was used

RESULTS

The smokers had 1821 live birth children and the controls 1823 There was no statistically significant difference in the number of boys and girls born to the different smoking groups nor was there any difference in the number of twin pregnancies

Table 2 *Postneonatal mortality from 28 days to 5 years among the smokers and their controls*

Series is divided into social groups according to father s occupation

Social group	Smokers Deaths (*N*)	Alive after neonatal period (*N*)	Per 1000	Controls Deaths (*N*)	Alive after neonatal period (*N*)	Per 1000
I+II	?	311	6 4	2	393	5 1
III+IV	13	1 167	11 1	4	1 053	3 8
Farmers	?	153	13 1	0	197	0 0
Not known[a]	3	165	18 2	1	157	6 4
Total	?0	1 796	11 1	7	1 800	3 9

Based on the social standing of the occupation in question (36)
Mostly mother unmarried

Perinatal mortality in smokers and controls by social groups is presented in Table 1 The difference between groups was not statistically significant either for the total groups or sub groups The effect of the slightly different distribution into social groups among the smokers and controls was checked by repeat ing the calculation using the mortality rate of the controls and the total number of cases among the smokers for each social group The perinatal mortality was thus 2? 4 per thousand instead of the true figure 23 9 per thousand in the controls the difference being without significance The mortality rate was higher among the heavy smokers than in the other groups 32 6 for the heavy smokers and 25 7 per thousand for the light smokers but the dif ference was without statistical significance

Table 2 depicts the *postneonatal mortality* in these groups The difference between the total groups of smokers and controls was statis tically almost significant ($p<0\ 05$) and the dif ference was noticeable in each social group ex cept the highest When the postneonatal mor tality was calculated for the controls for the case in which the distribution into social groups would be the same as among the smokers the result was not affected being 3 9 per thou sand The figures for heavy and light smokers were about the same 13 0 and 11 1 per thou sand respectively

Visits to the doctor and *hospital admissions* to any hospital in the area were recorded up to age of one year for 1 554 children of smokers and 1 560 children of controls As may be seen from the results presented in Table 3 the chil

Table 3 *Visits to the doctor and hospital admissions at the age of under one year by smoking groups*

Visits to all hospitals in the study area are included

	Number of live births	Visits to the doctor Children (*N*)	Visits (*N*)	Visits mean for group	Hospitalizations Children (*N*)	Visits (*N*)	Visits mean for group
Light smokers	1 30?	71?	986	0 76	??3	292	0 2
Controls	1 300	672	977	0 71	190	253	0 19
Heavy smokers	??	160	?10	0 83	70	98	0 39
Controls	?58	1 0	157	0 61	33	38	0 15
All smokers	1 554	87	1 196	0 77	293	390	0 ?5
Controls	1 558	79?	1 084	0 69	?23	291	0 19

$p>0\ 05$ $p<0\ 05$ $p<0\ 01$ * $p<0\ 001$

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RESULTS

The smokers had 1821 live birth children and the controls 1823 There was no statistically significant difference in the number of boys and girls born to the different smoking groups nor was there any difference in the number of twin pregnancies

Table 6 *Incidence of diseases during the first 5 years among the children of the smokers and the controls*

Incidence is based on all diagnoses given during hospital admissions in pediatric departments

	Smokers (*N* 1821)		Controls (*N* 1823)	
Diagnosis	*N*	Per thousand live births	*N*	Per thousand live births
Infective and parasitic dis	118	64 8	103	56 5
Neoplasms	3	1 6	5	2 7
Endocrine nutritional and metabolic dis	23	12 6	21	11 5
Dis of the blood and bloodforming organs	85	46 7	48	26 3
Mental disorders	5	2 7	11	6 0
Dis of the nervous system and sense organs	90	49 4	52	28 5
Dis of the circulatory system	3	1 6	6	3 3
Dis of the respiratory system	311	170 8	179	98 2
Dis of the digestive system	39	21 4	34	18 7
Dis of the genito urinary system	53	29 1	33	18 1
Dis of the skin and subcutaneous tissue	41	22 5	15	8 2
Dis of the musculoskeletal system and connective tissue	11	6 0	4	2 2
Congenital anomalies	44	24 2	51	28 0
Causes of perinatal morbidity and mortality	176	96 6	128	70 2
Symptoms and ill-defined conditions	67	36 8	54	29 6
Accidents poisonings and violence	39	21 4	25	13 7
Examination and investigation	14	7 7	12	6 6
Total	1 122	616 1	781	428 4

$p<0\ 05$ $p<0\ 01$ $p<0\ 001$

groups as those of the smokers 191 5 instead of 188 2 children per thousand had been hospitalized among the controls the difference being without significance

The mean duration of hospitalization was 14 0 days for the children of the smokers and 11 1 days for those of the controls the difference being statistically highly significant The mean duration of hospitalizations among the heavy smokers was 14 9 days among the light smokers 13 7 days

Table 6 presents the *frequency of all disease groups* diagnosed in children s departments *per thousand live births* among the children of smokers and of their controls If the child had been in hospital more than once for the same disease it was counted only once Respiratory and skin diseases were more frequent among the smokers than among the controls the difference being statistically highly significant ($p<0\ 001$) and the children of smokers also had blood and nervous diseases and disease of the newborn period more often the difference being statistically significant ($p<0\ 01$) Among the respiratory diseases the ratio of the incidence among the smokers to that of the controls was 2 2 in pneumonia 1 8 in bronchitis and 1 5 in others such as acute nasopharyngitis sinusitis etc In addition two cases of pulmonary atelectasis and one of empyema were recorded among the smokers but none among the controls

Among the skin diseases the ratio of the incidence among the smokers to that among the controls was 4 7 in eczema and urticaria and 2 0 in infectious diseases of the skin and subcutaneous tissue and other diseases of this category

Under the age of one year the difference in the diseases was in general greater between the heavy smokers and their controls than between the light smokers and their controls but this was no longer true for the age from one to five

When the *main causes* of hospitalization in pediatric departments per thousand live births was calculated for both groups a statistically highly significant difference ($p<0\ 001$) was

Table 4 *Hospital admissions by smoking groups*

Admissions to the pediatric departments of the four central hospitals in the study area

	Alive at age		Age under one			Age one to five		
	0 year (*N*)	1 year (*N*)	Children (*N*)	Visits (*N*)	Mean for group	Children (*N*)	Visits (*N*)	Mean for group
Light smokers	1 518	1 486	207	263	0 17	209	320	0 22
Controls	1 520	1 499	170	209	0 14	154**	231*	0 15*
Heavy smokers	303	295	63	90	0 30	40	73	0 25
Controls	303	299	22***	25***	0 08***	42	50*	0 17
All smokers	1 821	1 781	270	353	0 19	249	393	0 22
Controls	1 823	1 798	192***	234***	0 13	196 *	281 **	0 16 *

$^{-}p>0\ 05$ $^{*}p<0\ 05$ $^{**}p<0\ 01$ * $p<0\ 001$

dren of the smokers visited the doctor more often and were more often admitted to the hospital than those of the controls the difference in the number of children visits and means for the groups having varying degrees of statistical significance It is also clear that the differences are chiefly attributable to the effect of the heavy smokers

Hospital admission to the pediatric departments of the four central hospitals in the area were recorded for all children born to the smokers and controls (1 821 and 1 823) The results are presented in Table 4 separately for age under one year and for age from one to five The children of the smokers had significantly more hospital admissions than those of the controls The difference was more due to the group of heavy smokers in the case of children of under one year but this was no longer true for the age from one to five years

The difference between the smokers and the controls in the percentage of children *hospitalized during their first 28 days* was almost significant ($p<0\ 05$) the figures being 7 6% in former and 5 9% in the latter group

Table 5 presents the number of children *hospitalized* per thousand live births among the smokers and controls *by social groups* The children of each social group were more often hospitalized if the mother smoked during pregnancy than if she did not with the exception of the group of more well to do farmers In the case that the total number of cases among the controls had distributed into social

Table 5 *The children of the smokers and their controls admitted to pediatric departments during the first 5 years of life*

Series is divided into social groups according to father s occupation

	Smokers (*N* 1821)		Controls (*N* 1823)	
Social group	Hospitalized children	Per thousand live births	Hospitalized children	Per thousand live births
I	14	202 9	20	170 9
II	58	234 8	44	156 6
III	185	265 0	137	213 4
IV	114	236 0	85	201 4
Farmers I[b]	5	102 0	12	131 9
Farmers II	29	271 0	23	209 1
Not known[c]	43	256 0	22	137 5
Total	448	246 0	343	188 2

Based on the social standing of the occupation in question (36)

[b] Land under cultivation [illegible] hectares or over

[c] Mostly mother unmarried

and metabolic diseases The frequency of respiratory diseases was 435 3 per thousand among the children of the smokers and 390 2 per thousand among the controls

45 children of smokers and 30 children of controls *died* before the age of 5 years *The causes of death* calculated per thousand live births are seen in Table 7 38 children of the smokers and 29 children of the controls were in hospital at the time of death The recorded cause of death was based on autopsy in 59 cases Among the 8 cases who were not in hospital at the time of death 4 were accidental deaths and 2 died of pneumonia 1 of meningo coccal septicaemia and 1 of cerebral palsy The disease groups which were commonest among the children of smokers on the basis of their diagnosis at children s hospitals were on the whole also the more frequent causes of death in these cases than among their con trols but the differences were not statistically significant

DISCUSSION

The number of smokers in this series is con siderably lower than in most other series re ported Goldstein (12) has tabulated the figures for the six largest series commonly referred to in connection with maternal smoking and its effect on the foetus and the child having as the lowest percentage of smokers at the be ginning of pregnancy the 21% of this series (?6) while the figures in the other series vary from 32 to 54% During the two first months of pregnancy the number of smokers in this series had dropped to 15 1%

The average *number of cigarettes smoked* in this series was probably also lower than in the other series even though it is not easy to make comparisons with other studies because of the different criteria used for the classification into light and heavy smokers For example the Ontario Perinatal Mortality study (20) used the maximum number smoked per day any time during pregnancy the light smokers being those who smoked less than one packet and the heavy smokers those who smoked more than one packet Thus the group classified in this series as heavy smokers may show more similarity with the light smokers than the heavy smokers in the Ontario study

According to Butler & Goldstein (4) *aban donment of smoking* by the fourth month *of pregnancy* gives a mortality risk and expected birth weight comparable to those for mothers who are not smokers The findings of Donovan (10) are however not in full agreement with this In an earlier analysis of this series (26) it was shown that perinatal mortality was not significantly higher among smokers than among non smokers when the group of smokers was taken to include all those who smoked regularly at the beginning of the preg nancy In view of the findings by Butler & Goldstein mentioned above and since those who stopped smoking during pregnancy in this series were also known to be on average lighter smokers than those who continued it was reasonable to exclude the 519 mothers who did not smoke after the second month of pregnancy who formed about one fifth of the original group in order to highlight the effects of maternal smoking On the other hand a separate analysis was also made of those who had only stopped smoking during the last three months of pregnancy and even this subgroup showed similar figures for postneonatal mor tality and morbidity up to the age of 5 to those of their controls (29)

In the choice of the controls great attention was paid to obtaining the best possible match with the study group in respect of the place of residence This was done because one of the most prominent among the many differences between the smokers and non smokers was the concentration of the smokers in the popu lation centres and in this extensive study area —160 000 km² (26)—the regional differences in childhood mortality and the use of the health care services are in many respects more im portant than the social class differences be tween the families (27 28) Since the controls also had to be similar to the smokers in respect

Table 7 *Causes of death at age under five years among the children of the smokers and their controls*

Diagnosis	Smokers (*N* 1821) *N*	Smokers (*N* 1821) Per thousand live births	Controls (*N* 1823) *N*	Controls (*N* 1823) Per thousand live births
Infective and parasitic dis	4	2.20	0	0.00
Neoplasms	2	1.10	1	0.55
Mental disorders	0	0.00	1	0.55
Dis of the nervous system and sense organs	4	2.20	1	0.55
Dis of the respiratory system	5	2.75	1	0.55
Dis of the digestive system	0	0.00	1	0.55
Dis of the genito urinary system	0	0.00	0	0.00
Congenital anomalies	4	2.20	7	3.84
Causes of perinatal morbidity and mortality	23	12.63	16	8.78
Accidents poisonings and violence	3	1.65	2	1.10
Total	45	24.71	30	16.47

[a] Down's syndrome

found only in the case of respiratory diseases and an almost significant difference ($p<0.05$) in the case of skin diseases. The more frequent hospitalization of the children of smokers because of respiratory diseases was clearest below the age of one but also existed at the age of one to five, the difference between the smokers and controls at that age being almost significant ($p<0.05$). Again the children of the heavy smokers were more affected than those of the light smokers under the age of one but not at the age of one to five.

Since the higher frequency of other disease groups than respiratory diseases diagnosed among the children of smokers might be the result of more frequent hospitalization in the case of respiratory diseases, the differences in the frequencies of skin, blood and nervous diseases and sense organs and diseases of the newborn period between the smokers and their controls were also calculated excluding those hospitalizations in which the main diagnosis was respiratory disease. In this case the difference in skin diseases was statistically significant ($p<0.01$), those in diseases of the newborn period and nervous diseases almost significant ($p<0.05$) and that in blood diseases without significance.

The hospital visits were studied *by seasons of the year* taking the months from November to March as winter, from May to September as summer and the rest combined as the spring and autumn period. The difference between the smokers and the controls in the mean number of hospitalizations per child was highly significant in summer, significant in winter and almost significant in spring and autumn period. No clear trend was found for hospital admissions due to respiratory diseases to be any more accentuated among the children of smokers during the winter, even though the absolute figures for hospital admissions because of respiratory diseases were certainly greater during the winter both for the smokers and the controls.

In the diagnoses given during admission to any hospital in the area in the case of children under one year of age a highly significant difference ($p<0.001$) was found between the children of the smokers and those of the controls in respiratory diseases, the frequencies being 88.2 and 35.7 per thousand respectively. In blood diseases the differences was almost significant ($p<0.05$).

In causes of visits to the doctor under one year of age the children of the smokers had a statistically almost significantly ($p<0.05$) higher frequency for respiratory diseases, blood diseases, diseases of genitourinary system and the group of endocrine, nutritional

and metabolic diseases The frequency of respiratory diseases was 435 3 per thousand among the children of the smokers and 390 2 per thousand among the controls

45 children of smokers and 30 children of controls *died* before the age of 5 years *The causes of death* calculated per thousand live births are seen in Table 7 38 children of the smokers and 29 children of the controls were in hospital at the time of death The recorded cause of death was based on autopsy in 59 cases Among the 8 cases who were not in hospital at the time of death 4 were accidental deaths and 2 died of pneumonia 1 of meningo coccal septicaemia and 1 of cerebral palsy The disease groups which were commonest among the children of smokers on the basis of their diagnosis at children s hospitals were on the whole also the more frequent causes of death in these cases than among their con trols but the differences were not statistically significant

DISCUSSION

The number of smokers in this series is con siderably lower than in most other series re ported Goldstein (12) has tabulated the figures for the six largest series commonly referred to in connection with maternal smoking and its effect on the foetus and the child having as the lowest percentage of smokers at the be ginning of pregnancy the 21% of this series (26) while the figures in the other series vary from 32 to 54% During the two first months of pregnancy the number of smokers in this series had dropped to 15 1%

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In this series a clearly higher number of visits to the doctor admissions to any hospital in the area and admissions to children s departments was found among the children of th smokers this being most obvious under the age of one year (Tables 3 and 4) The difference between the children of the smokers and those of the controls was *highest in admissions* to children s departments and lowest in visits to the doctor (Tables 3 and 4) a fact which supports the theory that the difference was clearest in the more severe diseases as was shown to be true for the *respiratory diseases* The longer mean duration of hospital admissions among the children of smokers a finding departing from that of Harlap & Davies (13) is most probably also an index of the higher degree of severity of their diseases

The hicher diseases frequency in the children of the smokers as calculated from the hospital admissions was clearest in the case of *respiratory diseases* this being in agreement with most other investigations (5 7 13 18) The children of the smokers in this series were also admitted to the children s departments more often than the controls because of skin diseases the difference being clearest in diseases of the allergic type the most likely cause for this being the allergic effect of tobacco smoke in environment

Concerning the diagnoses which were not the main causes of hospital admission but given during hospitalizations it is difficult to judge if any real difference exists between the groups It may be a question either of children taken into hospital for one reason being more likely to acquire other diagnoses more frequently than those not taken into hospital or of children suffering from one disease being more prone to others The latter is true for example in the case of iron deficiency anemia the most common blood disease among Finnish children for children with frequent or serious respiratory infections are also more disposed to this type of anemia On the other hand the lesser degree of significance is not necessarily a sign of a smaller difference between the groups but may also reflect the smaller number of cases in the group which is the case when only some of the hospital admissions are concerned

The difference in respiratory diseases between the children of the smokers and of the controls was not so clear in the causes of visits to the doctor as in the causes of hospital admissions Part of the explanation at least may be found in the fact that the difference between the groups is clearest in the more severe diseases However the most frequent causes *of out* patient visits among the children of the smokers were mainly found in the same diseases groups as in the diagnoses given in the children s departments (Table 6)

A clear *dose related effect* under the age of one year was found both in the numbers of visits to the doctor and admissions to hospital

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The method of collecting the data on admissions to children s departments differed from that used for ascertaining admissions to any hospitals in the area or out patient visits the former being recorded directly from the hospital records and the latter two being collected by questionnaire from the child welfare centres It is probable that in the latter two cases some under estimation may exist but this would affect the study group and controls similarly The effects of regional differences were carefully eliminated when the control was chosen and the maternal smoking was not indicated in any phase of the follow up study 91 5% of the children in the northern province and 95 4% in the southern province were registered at a child welfare centre before the age of one in 1967 (23)

The studies most commonly referred to in which no adverse effect of maternal smoking on *perinatal mortality* is found are those of Jarvinen & Osterlund (15) O Lane (17) Peterson et al (25) Underwood et al (37 38) Yerusalmy (39 40) and the present series (26) Sets of data showing that maternal smoking may also have an adverse effect on perinatal mortality have become more numerous however, the best known investigations being those of Andrews & McGarry (1) Bailey (2) Butler et al (3 4) Comstock et al (8 9) Fabia (11) Russel et al (32) Meyer et al (19–21) and Rush & Kass (31)

In order to explain the contradictory findings on the effect of maternal smoking on perinatal mortality it has been suggested that smoking is more harmful to the infants of some groups of women than others (19 20) In the Ontario Perinatal Mortality Study Meyer et al (20) found that the increased risk of perinatal mortality due to maternal smoking was low if the mother was young of low parity non anemic and smoked lightly but high if the mother was of high parity of public hospital status had previous low birth weight infants or had a low hemoglobin level Similarly Rush & Kass (31) found that black smokers had a perinatal mortality rate considerably higher than other groups while among white mothers the effect of smoking was of lesser magnitude In Washington County study (8) the higher mortality rate caused by maternal smoking was most marked among families who ranged low on socio economic characteristics and the infants of primiparas and young mothers were less likely to suffer in this way

Even though the lowering effect of maternal smoking on the birth weight in this series was clear (26 29) the perinatal mortality was no higher among the smokers and no clear trend for maternal smoking to be more injurious in the lower social groups was found (Table 1) One possible explanation for this deviant result is the small number of heavy smokers in the series and the fact that the smokers were in general young low parity women (30)

The finding that maternal smoking increased *childhood mortality after the perinatal period* is similar to that of Comstock & Lundin (8) the two highest social groups being least affected (Table 2)

In contrast to the dissimilar findings concerning maternal smoking and perinatal mortality in different series the few investigations made on the effect of maternal smoking in *childhood morbidity* mainly agree well especially for children under one year of age

In the study by Cameron et al (5) based on telephone interviews with 727 Detroit metropolitan families it was found that the presence of tobacco smoke in the environment was associated with poorer physical health in children aged 16 or less respiratory diseases being the most common causes of illness Harlap & Davies (13) have investigated admissions to hospital in West Jerusalem infants during their first year of life noting that the infants of mothers who smoked during pregnancy 9 2%

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and in the frequencies of the diseases the children of heavy smokers being more seriously affected The explanation for the fact that this was no longer true for children of the age of one to five may lie in the greater resistance of this age group but also be related to the fact that maternal smoking habits were recorded only during the pregnancy and overlapping has occurred between the groups of heavy and light smokers and even between the smokers and non smokers in the course of the years On the other hand the small number of heavy smokers in the series makes this group more easily effected by random variation than the five times larger group of light smokers

In contrast to the study on Jerusalem infants by Harlap & Davies (13) no clear trend was found in this series for hospital admission caused by maternal smoking to be more frequent in winter Part of the explanation at least may lie in the fact that the healthiest period for Finnish children is not exactly identical with the summer months but is located on average during later summer and early autumn May and June still being quite busy times in pediatric practise

Maternal smoking was recorded here concerning pregnancy but not later However most mothers who do not stop smoking during pregnancy do not do so after it either as discussed more fully in another context (29) Paternal smoking was not recorded in this study as is the case with most mother–child studies However mothers spend more time at home on average than do fathers especially during their children's early years and on the other hand it is obvious that it will not be any more common for the father to smoke in families where the mother does not than in those whose she does

In order to explain the differences found between the children of the smokers and the controls the following hypotheses can be presented First it can be assumed that even if the controls were chosen as carefully as possible from the five times larger group of non smokers, they would not differ from these only in their smoking habits but in several other respects e g in their biological characteristics in their manner of taking care of their own health and that of their children in their dietary habits frequency of breast feeding and so on In fact some doubts are still expressed as to whether any true causal relationship exists between maternal smoking and increased perinatal mortality or reduced birth weight (10 12 35) At the same time however Goldstein (12) has shown that Various attempts to falsify the causal hypotheses have failed leaving us with good reason for acting as if smoking really did cause a decrease in birthweight and an increased risk of perinatal mortality

If we accept the causal theory also in respect of the later childhood period we still have to judge which is more important the foetal period or the infancy period For the diseases in which the children of the smoker differed most from the others in this series the respiratory and skin diseases it seems reasonable that the smoke in the environment should be most important However the importance of other contributing factors such as lower birth weight has not yet been analysed thoroughly

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OESOPHAGEAL VARICES AS A LATE COMPLICATION TO NEONATAL UMBILICAL VEIN CATHETERIZATION

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ABSTRACT Birk Lauridsen U Enk B and Gammeltoft A (Department of Paediatrics and Surgical Department D Gentofte Hospital Gentofte Denmark) Oesophageal varices as a late complication to neonatal umbilical vein catheterization Acta Paediatr Scand 67 633 1978.—Three recent cases are presented Haematemesis in children with splenomegalia and a history of neonatal umbilical vein catheterization should involve careful examination for oesophageal varices due to portal hypertension

KEY WORDS *Oesophageal varices portal hypertension neonatal umbilical vein catheterization*

Portal hypertension as a late complication to neonatal umbilical vein catheterization is a rarely described condition Recently Junker et al (5) have described the first Scandinavian case stating that they had found only 38 cases with this possible etiology in the literature

In 1976 three children were admitted to the Department of Paediatrics Gentofte Hospital because of haematemesis due to oesophageal varices All children had been exposed to neonatal umbilical vein catheterization

CASE HISTORIES

Case 1

Now 9 years old girl born 5 weeks before term following a normal pregnancy Birth weight 1760 g length 43 cm Apgar score 7 and 10 after 1 and 5 min respectively Immediately after delivery admitted to paediatric department *due to* Respiratory Distress Syndrome and treated with oxygen penicillin glucocorticoids and—through an umbilical vein catheter—infusion of glucose 5% and a total of 10 mEq of sodium bicarbonate On the fourth day of life the umbilical vein catheter was reestablished and 3 exchange transfusions were given because of hyperbilirubinaemia without signs of blood group incompatibility Between the exchange transfusions glucose 10% 70 ml was infused *for days* The umbilical vein catheter was removed after a total of 6 days After the age of 6 months the patient developed increasing splenomegalia and when 4 years old thrombopenia and leucopenia On the suspicion of splenic vein thrombosis splenectomia was performed when the patient was 7 years 4 months old The spleen was enlarged (345 g) but histologically normal During the operation no signs of splenic vein thrombosis or increased pressure of the mesenterial vessels were found and the liver was normal After splenectomy thrombocyte and leucocyte counts were normalized

At 9 years one month the patient was admitted to the Department of Paediatrics Gentofte Hospital on account of haematemesis Due to influenza like symptoms she had received acetylsalicylic acid 150 mg twice during the preceding 2 days On admittance she was preshocked and treated with 4 l blood during the first 4 hours Gastroscopy was normal except for blood clots X ray examination of oesophagus and stomach however showed oesophageal varices Percutan transhepatic portography revealed normal intrahepatic portal vessels but no opaque medium entered the portal vein X ray diagnosis portal vein thrombosis Laboratory examination showed normal liver function tests The oesophageal varices were treated with repeated injections of sclerosant fluid (aethoxysclerol 3%)

Case 2

Now 3 years old boy born 6 weeks before term following a normal pregnancy except for vaginal bleeding Birth weight 1050 g length 42 cm Apgar score 10 after 1 min Placenta was without infarcts Immediately after delivery admitted to children s hospital for prematurity and dysmaturity Because of hypoglycaemia treated with infusion of glucose through an umbilical vein catheter from the second day *of life* (Besides glucose 5% glucose 10% 300 ml was infused during 4 days) On the 6th day of life one exchange transfusion was given because of hyperbilirubinaemia without signs of blood group incompatibility The umbilical vein catheter was removed after 5 days At two months of age the child received blood transfusions due to anaemia of unknown origin When four

months old readmitted because of haematemesis and treated with blood transfusion X ray examination of oesophagus and stomach as well as oesophagoscopy were normal At six months again admitted because of severe haematemesis On the suspicion of bleeding Meckel s diverticulum an explorative laparotomy was performed with ligature of bleeding varicose veins in fundus ventriculi The enlarged spleen (41 g) was removed Histological examination normal spleen During the operation the portal vessels were considered normal

When 3 years 10 months the patient was admitted for haematemesis to the Department of Paediatrics Gentofte Hospital The child had received 250 mg acetylsalicylic acid twice during the preceding 24 hours After blood transfusions X ray examination of oesophagus and stomach suggested oesophageal varices a finding confirmed by oesophagoscopy which demonstrated varices 16–18 cm from the teeth The varices were treated with repeated injections of aethoxysclerol The liver was not enlarged and except for transitory elevated serum alanine amino transferase and decreased prothrombine liver function tests were normal

Case 3

Now 8 years old girl born 2 weeks before term following a pregnancy complicated with preeclampsia Birth weight 1 780 g length 44 cm Apgar score 10 after 1 min Placenta contained 3 white infarcts Immediately after delivery admitted to paediatric department for dysmaturity Because of hypoglycaemia treated with glucose infused through an umbilical vein catheter (glucose 10% 335 ml glucose 15% 620 ml and glucose 50% 8 ml during 4 days) The catheter was reestablished on the 3rd day of life and removed after a total of 4 days because of infection of the umbilicus (staphylococcus aureus) treated with ampicillin At 5 years 8 months readmitted for melaena treated with blood transfusions X ray examination of the gastrointestinal tract was normal On the suspicion of bleeding Meckel s diverticulum explorative laparotomy was performed without revealing the source of the bleeding The patient was readmitted 7 years old for severe haematemesis after having received 150–300 mg acetylsalicylic acid shortly before admittance She was treated with blood transfusion and a new explorative laparotomy was performed with ligature of a bleeding vessel in the pylorus

Readmitted for haematemesis at 8 years 6 months and treated with blood transfusions X ray examination of oesophagus and stomach was normal Gastroduodenoscopia shortly after admission showed a superficial ulcer in the duodenal bulb but repeated gastroscopy 4 weeks later revealed pronounced varices in the distal 10 cm of oesophagus The patient was transferred to the Department of Paediatrics Gentofte Hospital where splenomegalia was found By splenoportography varicose veins were found around the stomach and distal part of oesophagus The splenic vein and the portal vein were found normal Biopsy of liver was normal Biopsy of spleen normal spleen with signs of congestion Because of three earlier severe bleeding episodes side to-side anastomosis of the portal vein and inferior vena cava was performed During the operation the spleen was found enlarged 4–5 times normal size and the portal vein was dilated with increased palpatory pressure

DISCUSSION

In our patients several factors may have been of importance for the development of portal hypertension *First* the position of the catheter tip was controlled by X ray in one patient only where the catheter tip was located in the portal sinus If the catheter tip is located in the umbilical vein portal sinus or portal vein the risk for development of hepatic necrosis and portal vein thrombosis will be increased (8) Therefore most authors recommend passing the catheter tip through the ductus venosus to the inferior vena cava near the right atrium which according to Kitterman et al (6) is possible in about 60% of the cases However Kunaud (7) recommends passing the catheter tip to the internal end of the umbilical vein or to the portal sinus only because—in his opinion—by deeper insertion the catheter more frequently pass into the portal vein than through the ductus venosus into the inferior vena cava *Second* in our patients hypertonic solutions of glucose were infused while in one patient sodium bicarbonate was also given through the catheter Infusion of hypertonic or alkaline solutions should—if possible—be avoided due to the risk for development of inflammatory and thrombotic lesions (6) *Third* the patients had catheters in 4–6 days Larroche (8) found venous thrombosis in all patients with catheters left in situ for more than 48 hours The duration of the catheterization in our patients is in accordance with the three cases later developing portal vein thrombosis described by Levy et al (9) *Fourth* one patient had umbilical infection which may result in portal thrombophlebitis (2 10) Daschner et al (1) found infection in 23 4% of 125 umbilical venous catheters while the incidence of septicaemia was 3 2% increasing if the catheter remained in situ more than 4 days Our patients received prophylactic antibiotics but the value of this practice is ques

tionable (1 6) In two of our patients the catheters were reestablished after dropping out this may have increased the risk for development of infection

Reviewing the 38 hitherto published cases of portal hypertension following umbilical vein catheterization Juncker et al (5) found the onset of haematemesis from the age of seven months to 5 years while splenomegalia usually was demonstrated earlier (3 months to 3 years 3 months) One patient (case 2) had early onset of symptoms while haematemesis occurred late in cases 1 and 3 In cases 1 and 2 haematemesis occurred in spite of splenectomy performed 2–3 years before

In two of the patients earlier radiological and endoscopic examinations in connexion with haematemesis did not reveal oesophageal varices In our hospital however varices were demonstrated in all patients It thus appears that repeated investigations may be necessary to demonstrate the oesophageal varices In the first patient portal vein thrombosis was demonstrated by transhepatic portography Because of earlier splenectomy and only a single haematemesis anastomosis operation was omitted and she was treated with sclerosant injections The second patient probably had portal vein thrombosis as liver function was normal and as he had several bleeding episodes in spite of earlier splenectomy Due to his age (3 years) he was treated with sclerosant injections In the third patient splenoportography revealed normal portal and splenic veins Therefore a porta caval anastomosis operation was performed on the suspicion of hepatic obstruction (in spite of normal liver function tests and liver biopsy)

CONCLUSION

Haematemesis in children with splenomegalia and a history of neonatal umbilical vein catheterization should involve careful examination of oesophageal varices due to portal hypertension Repeated examinations may be necessary in order to demonstrate the varices In cases of oesophageal varices hepatic obstruction (cirrhosis) should be excluded by liver function tests and liver biopsy The choice of surgical treatment depends on the result of splenoportography to evaluate the state of the veins in the portal system specially the site of a possible obstruction The treatment of splenic vein thrombosis is splenectomy (11) In cases of portal vein thrombosis and hepatic obstruction the treatment depends on the age of the child (12) younger children should be treated with sclerosant injections (4) while shunt operation is indicated in older children with severe bleedings It should be emphasized that splenectomy is indicated in portal hypertension due to splenic vein thrombosis only In cases of portal vein thrombosis or hepatic obstruction splenectomy precludes a possible later spleno-renal shunt operation (3)

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FOLLICULAR DEVELOPMENT IN OVARIES OF CHILDREN WITH DOWN S SYNDROME

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ABSTRACT Højager B Peters H Byskov A G and Faber, M (The Finsen Laboratory Finsen Institute Copenhagen Denmark) Follicular development in ovaries of children with Down s syndrome Acta Paediatr Scand 67 637 1978 —Ovaries of children with Down s syndrome were examined histologically in order to determine in what way the ovarian development differed from the normal Twenty six specimens obtained at autopsy at various ages between birth and 14 1/2 years were available and compared with ovaries from normal children of similar ages All ovaries from normal children were characterized by active follicle growth The ovaries of the children with Down s syndrome however showed absence or retardation of follicle growth Furthermore the number as well as the size of the antral follicles differed from those in the normal ovary The decrease of the number of small follicles occurs earlier in life in the ovaries of children with Down s syndrome than in the control The possible relationship between an abnormal ovarian growth pattern and hormonal imbalance is discussed

KEY WORDS Down s syndrome childhood ovaries

Several aspects of the development of children with Down s syndrome (trisomy 21) have been well investigated The developmental pattern varies widely and so does the sexual develop ment Studies on the ovarian development in Down s syndrome in childhood are rare There have however been some isolated reports in which the ovaries were found to be abnormal with a reduced total ovarian mass and little fol licular growth (5 22)

The purpose of this study was to examine the development of the ovaries in children with Down s syndrome and to compare these to the ovaries from normal children of similar ages

MATERIALS AND METHODS

All ovaries were obtained at autopsy Twenty six ovaries came from children with Down s syndrome The ages of the children varied from 2 days to 14 1/2 years (Table I) Ten children were under 7 months of age (group I) nine were between 5 months and 3 years old (group II) four ovaries came from 3 1/2 to 5 years old children (group III) Two were obtained from children at 7 to 10 years of age (group IV) while one came from a 14 1/ years old girl (group V)

Thirty two ovaries obtained from normal children at similar ages served as controls They had died in acci dents of after a short acute disease

The ovaries were fixed in Bouin s solution and em bedded in paraffin Thirty to 180 serially cut mid sections at 5 or 7 μ were prepared and stained with haematoxylin and eosin Heidenhain s azan or with Schiff's reagent The sections were examined at a microprojector

The follicles were divided into several groups (?3)

1 *The small follicles* These consist of oocytes which have not yet started to grow and are surrounded by a few flat granulosa cells They are resting (non growing) fol licles

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Table 1 *Ovarian development in 26 children with Down s syndrome and 32 normal children*

Age	No of cases	Follicle stage: Small follicles Calculated no Mean ± S E M (*a*)	Preantral follicles From (*b*)	Preantral follicles To (*b*)	Small antral follicles From (*c*)	Small antral follicles To (*c*)	Large antral follicles no From (*d*)	Large antral follicles no To (*d*)	Collapsed follicles and/or scars From (*e*)	Collapsed follicles and/or scars To (*e*)
Group I 2 days–2 months										
Control	10	1 587±325	+	+++	±	+++	2	6	0	+
Down s syndrome	10	684±290	0	++	0	++	0	1	0	+
Group II 5 months–3 years										
Control	13	1 337±291	+	+++	±	+++	1	8	+	+
Down s syndrome	9	818±207	±	++	0	++	0	1	0	+
Group III 3½ years–5 years										
Control	3	686±129	++	+++	++	++	1	5	+	+
Down s syndrome	4	64± 35	±	+	±	+	0	1	+	+
Group IV 7 years–10 years										
Control	4	749±158	+++	+++	++	++	4	8	+	+
Down s syndrome	2	125± 97	+	+	±	+	1	3	+	+
Group V 14½ years										
Control	2	182±114	+++	+++	++	++	10	12	+	+
Down s syndrome	1	20	±		±		2		+	
Total										
Control	32									
Down s syndrome	26									

follicles of the 3 areas was then determined and the total number of small follicles in that section estimated by

$$\frac{\text{mean number of small follicles}\times C}{K}$$

The mean number of small follicles was then calculated for each group The actual number of preantral and small antral follicles was not counted but an impression of their frequency was recorded varying from 0 to +++ 3) The number of large antral follicles however was counted in that section 4) The diameter of the largest follicle was calculated as the mean of 2 diameters measured at right angles The mean diameter of the largest follicles was determined for each group

Four stages of ovarian development were recognized (22)

1 *The quiescent ovary* showed little or no growth It contained small resting follicles However a single preantral follicle might be seen

2 *The ovary showing early growth* contained besides small follicles also preantral and a few small antral follicles not larger than 0 5 mm in diameter

3 *The ovary showing retarded growth* contained besides small resting follicles occasionally a preantral or a small and even a large antral follicle In addition a few collapsed follicles as well as scars of follicles might be present (Figs 2 and 4)

4 *The actively growing ovary* contained in addition to small resting and preantral follicles also many small and large antral follicles healthy as well as atretic The latter are characterized by having pyknotic granulosa cells necrotic oocytes collapsing cavity and scars of large follicles (14 15)

RESULTS

Control ovaries

All ovaries from normal children showed follicle growth Twenty eight of the thirty two ovaries were actively growing with all stages of growing and atretic follicles except preovulatory ones (Table 1) Four ovaries of group I showed early growth No ovaries were quiescent

The outer cortex was populated by small non growing follicles in all specimens (Figs 1 and 3) The number of small follicles decreased with advancing age The number of small follicles was 28–50% smaller in group IV

Stage of ovarian development			
Quiescent (*f*)	Early growth (*g*)	Retarded growth (*h*)	Active growth (*i*)
0 8	4 0	0 2	6 0
0 3	0 0	0 6	13 0
0 0	0 0	0 4	3 0
0 0	0 0	0 2	4 0
0 0	0 0	0 1	2 0
0 1	4 0	0 15	28 0

than in group I (Table 1*a*). Variations in the number of small follicles within the groups and from group to group, however, were large. The largest decrease occurred between group IV and group V, when their number decreased by 50 to 75%. The number of preantral and small antral follicles did not seem to vary with age (Table 1*b*, *c*). The number and the size of large antral follicles increased after the age of 10 years, however, before this age these parameters varied (Table 1*d*, Fig. 5).

Ovaries from children with Down's syndrome

None of the ovaries showed early or active growth (Table 1*g*, *i*). Eleven ovaries (42%) were quiescent. These were found in group I and II (Table 1*f*). All others (15 cases) showed retarded growth, i.e. only a few growing follicles as well as scars were present (Figs. 2

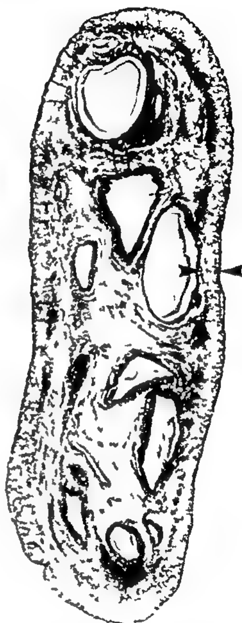


Fig. 1. Ovary of a 3 year-old normal child. Several small and large antral follicles as well as degenerating and collapsed follicles are present. The arrows indicate the outer cortex with small, non growing follicles. Cause of death: acute encephalitis. ×10

The number of small, resting follicles varied within the groups as well as from group to group. In all groups, however, the number was less than in the controls (Table 1*a*). Only 37–

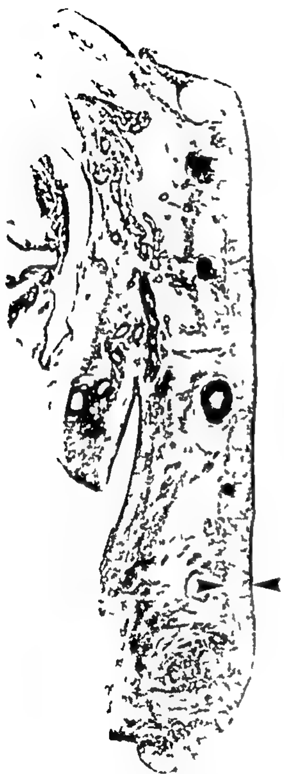


Fig 2 Ovary of a 3 year-old child with Down s syndrome Only few developing follicles are present The arrows indicate the outer cortex with small non growing follicles ×10



Fig 3 Ovary of a 7 year old child who died in a road accident The outer cortex is filled with small follicles The arrows indicate the outer cortex with small non growing follicles Numerous large antral follicles ×10

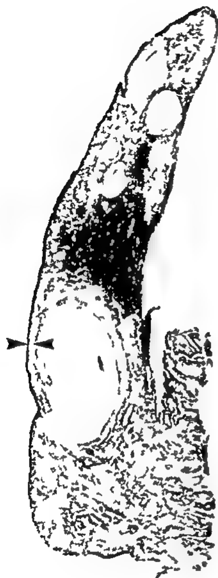


Fig 4 Ovary of a 7 year-old child with Down's syndrome The outer cortex has fewer small follicles and the number of antral follicles is reduced The arrows indicate the outer cortex with small non-growing follicles ×10

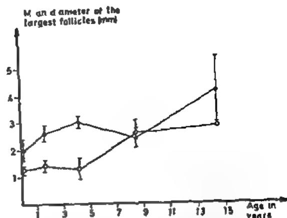


Fig 5 Mean diameter of the largest antral follicle in relation to age in ovaries of normal (●—●) and Down's syndrome (○—○) children

53% of a normal complement was found in the ovaries in group I and II After the age of 3 years a marked reduction was seen in group III to V only 10–25% of the expected number could be found (Table 1*a*)

In the ovaries showing retarded growth the number of developing follicles was low and seemed to be independent of age (Table 1*b* *c d*) The mean diameter of the largest follicle in the ovaries of group I to III was significantly smaller than in the controls ($t=12$ $p<0.01$) (Fig 5) After the age of 7 years the largest follicle was not different in size from that in the control ovaries

The ovaries of children with Down's syndrome often contained oocytes with two seemingly healthy nuclei (binuclear oocytes) These were especially frequent in group III to IV (Fig 6)

DISCUSSION

All ovaries obtained from normal children showed follicle growth and atresia This confirmed previous observations that the ovary is an actively growing organ throughout infancy and childhood (6 12 20 22 23 24 29)

All ovaries from patients with Down's syndrome were abnormal None of them were found to be early or actively growing ovaries It has previously been reported that children with Down's syndrome often showed a delay or inhibition of ovarian follicular growth (5 22) It was suggested that the disease might influence the normal development (23)

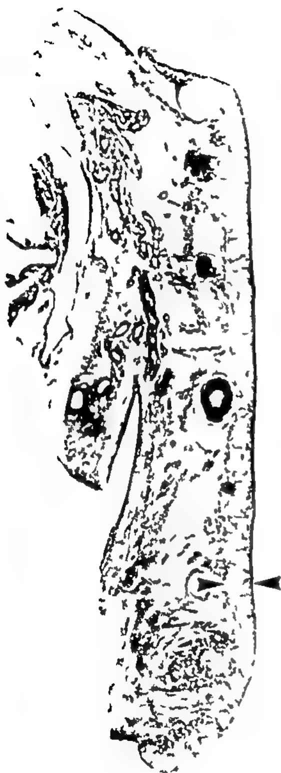


Fig 2 Ovary of a 3 year old child with Down s syndrome Only few developing follicles are present The arrows indicate the outer cortex with small non growing follicles ×10

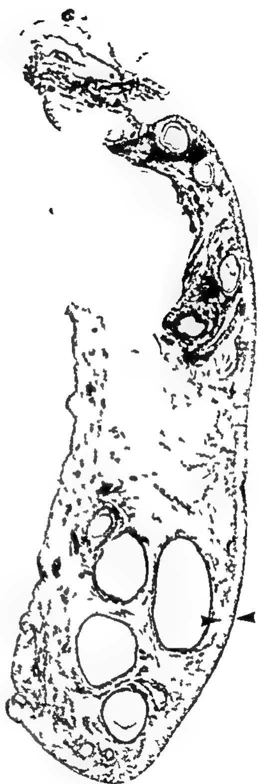


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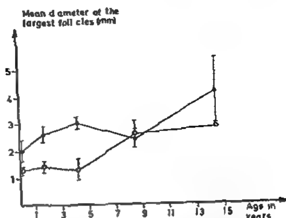


Fig 5 Mean diameter of the largest antral follicle in relation to age in ovaries of normal (●—●) and Down s syndrome (O—O) children

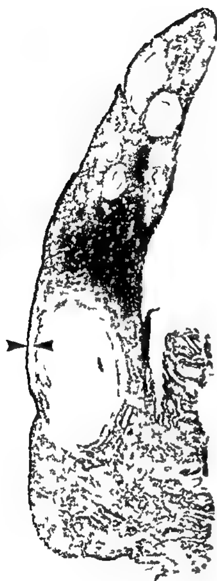


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Fig. 6. A small follicle with a binuclear oocyte in the ovary of a child with Down's syndrome. Age 7 years. ×950.

The present findings show that 42% of the ovaries of Down's syndrome children were quiescent with small resting follicles and no follicle growth. Even in the ovaries in which follicle growth was in progress, defects were found, as 1) early reduction in the number of small resting follicles and 2) reduced number and size of developing follicles.

The decrease in the number of small follicles with age in normal children found in the present study is in agreement with previous investigations (4, 7, 30). Compared to the findings in normal ovaries the number of small follicles in Down's syndrome is smaller in each group particularly after the age of 3 years.

The cause of the marked reduction early in life of the number of small follicles in Down's syndrome is not known.

The number of oocytes in ovaries of patients with chromosome aberrations is often reduced. In ovaries of trisomy 18 a marked reduction in the number of oocytes occurs during the first 3 weeks of life (1, 27). In the ovaries of women with Turner's syndrome the loss of oocytes are reported to occur at different ages. This reduction in the number of oocytes may occur already in ovaries of foetal and newborn children (8, 9, 11, 28). If oocytes are present at birth, they usually disappear during the years preceding puberty, and the organ becomes a streak gonad.

In 12 to 14 year old girls and young women with Turner's syndrome high values for gonadotrophins are usually seen (26), while the estrogen levels are thought to be subnormal (21).

Histological examinations of pituitaries from patients with Down's syndrome show abnormalities in the basophil cells of the anterior lobe (5), which might suggest a disturbance in gonadotrophin production (25).

Pituitary as well as ovarian hormones guide follicle growth. Whether the disturbed growth pattern in the ovaries of Down's syndrome children is primarily caused by pituitary dysfunction or by abnormal intraovarian hormone production can not be determined by the present material.

The occurrence of binuclear oocytes in small follicles in the ovaries of Down's syndrome children between the age of 3 years and 14 1/2 years might suggest an endocrine imbalance. Polynuclear oocytes are frequently found in early postnatal human ovaries, but they usually disappear at the age of six months (2, 3). Bacsich (3) suggested, that their presence in early life may be linked to the withdrawal of maternal gonadotrophin hormones or estrogens at the time of birth. Polynuclear oocytes have often been described in the ovaries of immature animals of many species (10, 13, 16, 17, 18, 19). Administration of estrogen reduced the number of polynuclear oocytes normally seen in the hamster ovary (17). It is suggested, that the persistence of binuclear oocytes in the cases of Down's syndrome might be the result of a low estrogen level.

Acta Pædiatr Scand 67 645–648 1978

EFFECT OF MATERNAL ANAEMIA ON THE PLACENTA AND THE NEWBORN INFANT

P N SINGLA S CHAND SUSHILA KHANNA and K N AGARWAL

From the Paediatric Haematology Unit Department of Paediatrics Institute of Medical Sciences Varanasi India

ABSTRACT Singla P N Chand S Khanna S and Agarwal K N (Paediatric Haematology Unit Department of Paediatrics Institute of Medical Sciences Varanasi India) Effect of maternal anaemia on the placenta and the newborn infant Acta Paediatr Scand 67 645 1978.—Foetal birthweight placental morphometry and maternal cord blood and placental haemoglobin and iron levels were studied in 69 anaemic mothers (haemoglobin <110 g/l) and 16 mothers without anaemia (haemoglobin ≥110 g/l) The birthweight placental weight and number of placental cotyledons were significantly reduced in the severely anaemic mothers (haemoglobin ≤60 g/l) and had direct relationships with the maternal haemoglobin levels However placental volume and surface area showed no constant relation to maternal haemoglobin The haemoglobin and iron levels in the cord blood and placental tissu were found to have linear correlations with the maternal haemoglobin levels The low levels of placental and cord serum iron in the severely anaemic mothers suggest that iron supply to the placenta and the foetus is affected in maternal anaemia and the foetus takes iron in direct proportion to the levels available in the mother

KEY WORDS Maternal anaemia placenta newborn infant

Surveys carried out in different parts of India indicate that more than 50% of women have nutritional anaemia in the later months of pregnancy mainly due to iron deficiency (5) Available evidence suggests that the foetus and the placenta quite effectively parasitize iron from the mother even when she is grossly deficient in this nutrient (7 9) Sturgeon (14) showed that the cord blood haemoglobin levels were similar in anaemic and non anaemic mothers Sisson & Lund (13) and Nhonoli et al (8) however observed a significant decrease in haemoglobin and serum iron in the cord blood of iron deficient mothers

The present study covers foetal birthweight placental morphometry and haemoglobin and iron levels in maternal blood cord blood and placental tissue in pregnancy anaemia

MATERIAL AND METHODS

Sixty nine sets of anaemic mothers (haemoglobin <110 g/l) newborn infants and placentae were selected at random from the Neonatology Unit Department of Paediatrics Institute of Medical Sciences Varanasi Another 16 sets with maternal haemoglobin 110 g/l or above at delivery served as controls All these mothers had singleton livebirths with gestation ranging from 37 to 41 weeks They had no racial cultural or environmental differences The mothers with preterm delivery antepartum haemorrhage toxaemia of pregnancy blood group incompatibility and systemic diseases likely to affect the foetal growth were excluded from the study

Maternal venous blood was taken during the first stage of labour and the samples of cord blood were collected from the placental end without milking the cord just after the second stage As soon as the placenta was delivered the umbilical cord was cut flush with the placental surface and the membranes were trimmed off Adherent blood clots were removed from the maternal surface of the placenta and the subchorionic vessels were emptied of blood by gentle pressure The placenta was blotted several times with filter paper and weighed

The tracings of the placental margins were made on clean paper and the surface area of the placenta was calculated from these tracings by using a planimeter The number of cotyledons was counted in each placenta The volume of the organ was obtained by the water displacement method A small piece weighing about 5 0 g was then cut from the maternal surface of the placenta at a distance of about 1 0 cm from the cord and preserved at −20°C for the estimation of placental haemoglobin and iron levels

Maternal and cord blood haemoglobin levels were

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Table 1 *Foetal birthweight and placental morphometry in relation to maternal haemoglobin (mean ±S D)*

Figures in parentheses indicate sample size

Group	Range of maternal haemoglobin (g/l)	Birth weight (g)	Placenta Weight (g)	Placenta Volume (ml)	Placenta Surface area (cm²)	Placenta No of cotyledons	Placental coefficient
I	≤60 (14)	2 187±444	341±71	329±61	207±47	14.8±2.0	0.16±0.07
II	61–85 (35)	2 627±403	386±75	378±[illegible]	226±43	18.0±2.6	0.15±0.03
III	86–109 (20)	2 642±386	389±84	390±87	232±35	18.4±2.0	0.15±0.07
IV	≥110 (16)	2 939±278	402±81	386±73	233±47	19.6±2.2	[illegible]14±0.07
Total observations (85)		2 617±441	383±72	372±82	225±51	18.1±3.3	0.15±0.07
p value							
I/IV		*<0.001*	*<0.05*	*<0.05*	*n s*	*<0.001*	<0.07
II/IV		<0.005	n s	n s	n s	<0.05	n s
III/IV		<0.02	n s	n s	n s	n s	n s
Correlation coefficient (*r*)		+0.4899	+0.2326	+0.1654	+0.2060	+0.4110	−0.2507
Regression coefficient (*b*)		*+8.3583*	*+0.6512*	*+0.5273*	+0.4099	*+0.0523*	*−0.0007*
p value		<0.001	<0.05	n s	n s	<0.001	<0.05

n s =not significant

estimated by the cyanmethaemoglobin method (4) Serum iron was estimated using the technique of Ramsay (10) Unsaturated iron binding capacity was determined by the method of Ressler & Zak (11)

The placental tissue preserved at −20 C was wiped with filter paper and 1.0 g of the tissue was homogenized with 5.0 ml of ice cold distilled water for 3 min using a Potter Elvehjem homogenizer fitted with a teflon pestle The haemoglobin was estimated in the homogenate by cyanmethaemoglobin method (4) and iron was determined according to the technique of Hallgren as modified by Routh & Agarwal (12)

Table 2 *Cord blood and placental haemoglobin and iron values in relation to maternal haemoglobin (mean ± S D)*

Figures in parentheses indicate sample size

Group	Range of maternal haemoglobin (g/l)	Haemoglobin Maternal blood (g/l)	Haemoglobin Cord blood (g/l)	Haemoglobin Placenta (g/kg of placenta)	Iron Maternal serum (μmol/l)	Iron Cord serum (μmol/l)	Iron Placenta (μmol/kg of placenta
I	≤60 (14)	47± 9	127±19	102±18	8.6±3.2	15.9±3.7	270.3± 30.4
II	61–85 (35)	77± 7	147±23	123±26	13.5±4.6	19.4±5.0	398.4± 71.6
III	86–109 (20)	99± 8	166±21	154±34	15.5±4.5	20.9±2.9	476.1±103.8
IV	≥110 (16)	123±12	187±18	207±29	15.8±6.5	21.7±6.4	531.6±109.7
Total observations (85)		86±26	155±28	143±36	13.6±5.3	19.6±5.0	417.1±118.1
p value							
I/IV			<0.001	<0.001	<0.001	<0.005	<0.001
II/IV			<0.001	<0.001	n s	n s	<0.001
III/IV			*<0.005*	*<0.001*	*n s*	*n s*	n s
Correlation coefficient (*r*)			+0.7308	+0.9599	+0.4977	+0.4128	+0.7791
Regression coefficient (*b*)			+0.8044	+1.3506	+0.1006	+0.0802	+3.3386
p value			<0.001	<0.001	<0.001	<0.001	<0.001

n s =not significant

RESULTS

The mean gestational age ranged between 39 2 and 39 6 weeks in the 4 maternal haemoglobin groups shown in Table 1 The groups were also comparable in terms of parity and absence of severe morbidity during pregnancy

Birthweight and placental morphometry

The birthweight placental weight placental volume and number of cotyledons in the severely anaemic mothers (haemoglobin ≤60 g/l were significantly less than those in the mothers without anaemia (haemoglobin ≥110 g/l) The placental surface area however remained unchanged in the various haemoglobin groups The cumulated data coefficients of correlation for birthweight placental weight and number of placental cotyledons taking maternal haemoglobin as an independent variable (x) were found to be significant The standard error test for testing the significance of correlation coefficients showed that the chances of such correlations arising due to sample fluctuation were less than 2% The equations of regression of birthweight (y_1) placental weight (y_2) and number of placental

Transferrin saturation	
Maternal serum (%)	Cord serum (%)
10 ?±4 4	4? 0± 9 9
15 1±6 3	46 3±11 ?
18 1±7 7	51 4±15 ?
19 1±7 8	51 8±13 3
16 4±7 5	47 8±1? 8
<0 001	<0 05
n s	n s
n s	n s
0 46 1	+0 3_56
0 135	+0 1611
<0 001	<0 01

Table 3 *Regression equations of cord blood and placental haemoglobin and iron on maternal haemoglobin (g/l)*

Parameter (y)	Equation of regression on maternal haemoglobin (x)	S E of the predicted value
Cord blood haemoglobin (g/l)	86 30+0 8044 x	2 12
Placental haemoglobin (g/kg of placenta)	26 55+1 3506 x	1 11
Maternal serum iron (μmol/l)	4 95+0 1006 x	0 51
Cord serum iron (μmol/l)	12 71+0 0802 x	0 50
Placental iron (*μmol/kg of* placenta)	130 8+3 3386 x	8 8?

cotyledons (y_3) on maternal haemoglobin (x) were found to be y_1=1 898 96+8 3583 x y_2= 327 17+0 6512 x and y_3=13 63+0 0523 x the standard errors of the estimates being 41 98 7 66 and 0 33 respectively No definite relationship was observed between the maternal haemoglobin and either the placental volume or the placental surface area (Table 1)

The placental coefficient (placental/foetal weight ratio) was significantly higher in the mothers with severe anaemia compared to the non anaemic mothers and was found to have a negative correlation with the maternal haemoglobin levels (Table 1)

Maternal cord blood and placental haemoglobin and iron levels

The cord blood and placental haemoglobin the maternal cord serum and placental iron and the maternal and cord serum transferrin saturation were significantly low in the mothers with severe anaemia and had linear correlations with the maternal haemoglobin levels (Table 2) The mean serum iron values in cord blood were significantly higher than in maternal blood in all the groups (p<0 001 in groups I II & III and <0 02 in group IV) The cord blood and placental haemoglobin and iron levels can be predicted from the regression equations given in Table 3

Table 1 *Foetal birthweight and placental morphometry in relation to maternal haemoglobin (mean ±S D)*

Figures in parentheses indicate sample size

Group	Range of maternal haemoglobin (g/l)	Birth weight (g)	Placenta Weight (g)	Volume (ml)	Surface area (cm²)	No of coty ledons	Placental coefficient
I	≤60 (14)	2 187±444	341±71	329±61	207±47	14 8±2 0	0 16±0 02
II	61–85 (35)	2 627±403	386±75	378±88	226±43	18 0±2 6	0 15±0 03
III	86–109 (20)	2 642±386	389±84	380±87	232±35	18 4±2 0	0 15±0 02
IV	≥110 (16)	2 939±278	402±81	396±73	233±47	19 6±2 2	0 14±0 02
Total observa tions (85)		2 617±441	383±72	372±82	225±51	18 1±3 3	0 15±0 02
p value							
I/IV		<0 001	<0 05	<0 05	n s	<0 001	<0 02
II/IV		<0 005	n s	n s	n s	<0 05	n s
III/IV		<0 02	n s	n s	n s	n s	n s
Correlation coefficient (*r*)		+0 4889	+0 2326	+0 1654	+0 2060	+0 4110	−0 2507
Regression coefficient (*b*)		+8 3583	+0 6512	+0 5273	+0 4099	+0 0523	−0 0002
p value		<0 001	<0 05	n s	n s	<0 001	<0 05

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estimated by the cyanmethaemoglobin method (4) Serum iron was estimated using the technique of Ramsay (10) Unsaturated iron binding capacity was determined by the method of Ressler & Zak (11)

The placental tissue preserved at −20 C was wiped with filter paper and 1 0 g of the tissue was homogenized with 5 0 ml of ice cold distilled water for 3 min using a Potter Elvehjem homogenizer fitted with a teflon pestle The haemoglobin was estimated in the homogenate by cyanmethaemoglobin method (4) and iron was determined according to the technique of Hallgren as modified by Routh & Agarwal (12)

Table 2 *Cord blood and placental haemoglobin and iron values in relation to maternal haemoglobin (mean ±S D)*

Figures in parentheses indicate sample size

Group	Range of maternal haemoglobin (g/l)	Haemoglobin: Maternal blood (g/l)	Cord blood (g/l)	Placenta (g/kg of placenta)	Iron: Maternal serum (μmol/l)	Cord serum (μmol/l)	Placenta (μmol/kg of placenta
I	≤60 (14)	47± 9	127±19	102±18	8 6±3 2	15 9±3 7	270 3± 30 4
II	61–85 (35)	77± 7	147±23	123±26	13 5±4 6	19 4±5 0	388 4± 71 6
III	86–109 (20)	99± 8	166±21	154±34	15 5±4 5	20 9±2 9	476 1±103 8
IV	≥110 (16)	123±12	187±18	207±28	15 8±6 5	21 7±6 4	531 6±109 2
Total observa tions (85)		86±26	155±28	143±36	13 6±5 3	19 6±5 0	417 1±118 1
p value							
I/IV			<0 001	<0 001	<0 001	<0 005	<0 001
II/IV			<0 001	<0 001	n s	n s	<0 001
III/IV			<0 005	<0 001	n s	n s	n s
Correlation coefficient (*r*)			+0 7308	+0 9599	+0 4877	+0 4128	+0 7291
Regression coefficient (*b*)			+0 8044	+1 3506	+0 1006	+0 0802	+3 3386
p value			<0 001	<0 001	<0 001	<0 001	<0 001

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Maternal serum (%)	Cord serum (%)
10 ±4 4	47 0± 9 9
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19 1±7 8	51 8±13 3
16 4±7 5	47 8±1 [illegible]
<0 001	<0 05
n s	n s
n s	n s
0 46 1	+0 3 56
0 135	+0 1611
<0 001	<0 01

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Parameter (y)	Equation of regression on maternal haemo-globin (x)	S E of the predicted value
Cord blood haemo-globin (g/l)	86 30+0 8044 x	2 17
Placental haemo-globin (g/kg of placenta)	76 55+1 3506 x	1 11
Maternal serum iron (μmol/l)	4 95+0 1006 x	0 51
Cord serum iron (μmol/l)	12 71+0 0807 x	0 40
Placental iron (μmol/kg of placenta)	130 28+3 3386 x	8 87

cotyledons (y_3) on maternal haemoglobin (x) were found to be y_1=1898 96+8 3583 x y_2= 327 17+0 6512 x and y_3=13 63+0 0523 x the standard errors of the estimates being 41 08 7 66 and 0 33 respectively No definite rela tionship was observed between the maternal haemoglobin and either the placental volume or the placental surface area (Table 1)

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The cord blood and placental haemoglobin the maternal cord serum and placental iron and the maternal and cord serum transferrin saturation were significantly low in the mothers with severe anaemia and had linear correlations with the maternal haemoglobin levels (Table 2) The mean serum iron values in cord blood were significantly higher than in maternal blood in all the groups (p<0 001 in groups I II & III and <0 02 in group IV) The cord blood and placental haemoglobin and iron levels can be predicted from the regres sion equations given in Table 3

Table 1 *Foetal birthweight and placental morphometry in relation to maternal haemoglobin (mean ±S D)*

Figures in parentheses indicate sample size

Group	Range of maternal haemoglobin (g/l)	Birth weight (g)	Placenta Weight (g)	Placenta Volume (ml)	Placenta Surface area (cm^2)	Placenta No of cotyledons	Placental coefficient
I	≤60 (14)	2 187±444	341±71	329±61	207±47	14 8±2 0	0 16±0 02
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Total observations (85)		2 617±441	383±72	372±82	225±51	18 1±3 3	0 15±0 02
p value							
I/IV		<0 001	<0 05	<0 05	n s	<0 001	<0 02
II/IV		<0 005	n s	n s	n s	<0 05	n s
III/IV		<0 02	n s	n s	n s	n s	n s
Correlation coefficient (*r*)		+0 4889	+0 2376	+0 1654	+0 2060	+0 4110	−0 2507
Regression coefficient (*b*)		+8 3583	+0 6512	+0 5273	+0 4099	+0 0523	−0 0002
p value		<0 001	<0 05	n s	n s	<0 001	<0 05

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Group	Range of maternal haemoglobin (g/l)	Haemoglobin Maternal blood (g/l)	Haemoglobin Cord blood (g/l)	Haemoglobin Placenta (g/kg of placenta)	Iron Maternal serum (μmol/l)	Iron Cord serum (μmol/l)	Iron Placenta (μmol/kg of placenta
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Correlation coefficient (*r*)			+0 7308	+0 9599	+0 4877	+0 4178	+0 7291
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Maternal serum (%)	Cord serum (%)
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19 1±7 8	51 8±13 3
16 4±7 5	47 8±1 [illegible]
<0 001	<0 05
n s	n s
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Maternal serum iron (μmol/l)	4 95+0 1006 x	0 51
Cord serum iron (μmol/l)	17 71+0 0809 x	0 50
Placental iron (μmol/kg of placenta)	130 28+3 3386 x	8 82

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I/IV		<0 001	<0 05	<0 05	n s	<0 001	<0 0?
II/IV		<0 005	n s	n s	n s	<0 05	n s
III/IV		<0 02	n s	n s	n s	n s	n s
Correlation coefficient (*r*)		+0 4889	+0 2326	+0 1654	+0 2060	+0 4110	−0 2507
Regression coefficient (*b*)		+8 3583	+0 6512	+0 5273	+0 4099	+0 0523	−0 000?
p value		<0 001	<0 05	n s	n s	<0 001	<0 05

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Figures in parentheses indicate sample size

Group	Range of maternal haemoglobin (g/l)	Haemoglobin Maternal blood (g/l)	Cord blood (g/l)	Placenta (g/kg of placenta)	Iron Maternal serum (μmol/l)	Cord serum (μmol/l)	Placenta (μmol/kg of placenta
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p value			<0 001	<0 001	<0 001	<0 001	<0 001

n s =not significant

Acta Pædiatr Scand 67 649–653 1978

VARIATIONS OF SERUM TESTOSTERONE ESTRADIOL BINDING GLOBULIN (TeBG) BINDING CAPACITY IN INFANTS DURING THE FIRST YEAR OF LIFE

J L CHAUSSAIN A BRIJAWI P GEORGES M ROGER
M DONNADIEU and J C JOB

*From the Centre d'Etudes sur la Croissance et l'Endocrinologie Infantile
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ABSTRACT Chaussain J L Brijawi A Georges P Roger M Donnadieu M and Job J C (Centre d Etudes sur la Croissance et l Endocrinologie Infantile Hopital Saint Vincent de Paul Paris France) Variations of serum Testosterone estradiol Binding Globulin (TeBG) binding capacity in infants during the first year of life Acta Paediatr Scand 67 649 1978 —Serum TeBG binding capacity was measured in 8 cord blood samples and in peripheral venous blood from 39 male and 31 female infants aged from 1 day to 1 year In cord blood TeBG binding capacities were low (1 27±0.3 μg/100 ml) with no sex difference In male infants TeBG binding capacities increased progressively from birth to 3 months before decreasing to reach the normal prepubertal level at 6 months Individual values ranged between 1 16 and 14 5 μg/100 ml and were significantly correlated with plasma testosterone (r=0 671 95% confidence limits 0 440 to 0 818 p<0 005) and estradiol (r=0 734 95% confidence limits 0 121 to 0 942 p<0 01) levels In female infants individual values ranged between 1 17 and 14 5 μg/100 ml without correlation with age or plasma estradiol level In male infants the data suggest a positive control of TeGB binding capacity by estrogens the negative effect of testosterone being delayed until after the 3rd month of age In girls the lack of correlation between TeBG and estradiol can probably be explained by rapid variations of plasma estradiol levels

KEY WORDS Infants TeBG

The binding capacity of serum TeGB is influenced by variations of sex steroid levels It is increased by estrogens and decreased by testosterone (13)

Spontaneous variations of plasma sex steroid levels in infants during the first year of life have been extensively studied According to Forest et al (6–9) plasma testosterone levels are elevated at birth in both sexes and diminish during the first week of life Then in males only they rise to a peak value at about three months of age before decreasing again to the prepubertal value which is reached at the age of six months Variations of plasma estradiol levels during this period were also reported In male infants after an initial decrease during the first week of life estradiol increases and reaches a peak at the end of the second month (2) In female infants occasional elevated estradiol levels are frequently found during the first two years of life (2 4)

The knowledge of these variations of plasma sex steroid concentrations suggested the possibility of related variations of TeBG binding capacity The aim of this study was to measure the serum TeBG binding capacity in infants of both sexes during the first year of life and to investigate the correlation of TeBG with plasma sex steroid levels

SUBJECTS AND METHODS

Serum TeBG binding capacity was measured in 1) cord blood samples from 4 boys and 4 girls 2) venous blood from 39 male infants aged 1 to 240 days 3) venous blood from 31 female infants aged 3 to 360 days

Plasma testosterone was measured in the venous blood

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DISCUSSION

Beischer et al (2) and Agboola (1) observed that maternal anaemia was associated with placental hypertrophy Similar observations at high altitudes by Kruger & Arias Stella (6) suggested that placental hypoxia was possibly responsible for this hypertrophy Surprisingly the results of the present study showed that the placental weight was significantly low in the mothers with severe anaemia The foetal birthweight and the placental volume were also significantly reduced in the severely anaemic mothers The high placental coefficient found in these mothers suggested that the weight of the newborn infant suffered more than the weight of the placenta in maternal anaemia

The reduced number of cotyledons in the placentae of mothers with severe anaemia indicated a lesser degree of septation and probably a greater proportion of functioning parenchyma (6) This could be an adaptation to a physiological stress resulting in an improvement of placental function and foetal well being

The present study showed a direct relation between the maternal and cord blood haemoglobin levels The mean cord blood haemoglobin was found to be markedly reduced in the mothers with moderate to severe anaemia These observations are in accordance with those reported recently by Nhonoli et al (8)

Although the foetus is an efficient parasite it is not clear whether the foetus takes the optimum amounts of iron or it takes amounts proportional to the levels available in the mother in pregnancy anaemia The recent work of Nhonoli et al (8) supports the latter possibility The linear relationships of the cord serum iron and transferrin saturation with the maternal haemoglobin found in this study further strengthen this view The low cord serum and placental iron levels in the severely anaemic mothers could be responsible for the poor iron stores in infants born to these mothers (3)

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ABSTRACT Chaussain J L Brijawi A Georges P Roger M Donnadieu M and Job J C (Centre d Etudes sur la Croissance et l Endocrinologie Infantile Hopital Saint Vincent de Paul Paris France) Variations of serum Testosterone estradiol Binding Globulin (TeBG) binding capacity in infants during the first year of life Acta Paediatr Scand 67 649 1978.—Serum TeBG binding capacity was measured in 8 cord blood samples and in peripheral venous blood from 39 male and 31 female infants aged from 1 day to 1 year In cord blood TeBG binding capacities were low (1 27±0.3 μg/100 ml) with no sex difference In male infants TeBG binding capacities increased progressively from birth to 3 months before decreasing to reach the normal prepubertal level at 6 months Individual values ranged between 1 [illegible] and 14 5 μg/100 ml and were significantly correlated with plasma testosterone (r=0 671 95% confidence limits 0 440 to 0 818 p<0 005) and estradiol (r=0 734 95% confidence limits 0 121 to 0 942 p<0 01) levels In female infants individual values ranged between 1 17 and 14 5 μg/100 ml without correlation with age or plasma estradiol level In male infants the data suggest a positive control of TeGB binding capacity by estrogens the negative effect of testosterone being delayed until after the 3rd month of age In girls the lack of correlation between TeBG and estradiol can probably be explained by rapid variations of plasma estradiol levels

KEY WORDS Infants TeBG

The binding capacity of serum TeGB is influenced by variations of sex steroid levels It is increased by estrogens and decreased by testosterone (13)

Spontaneous variations of plasma sex steroid levels in infants during the first year of life have been extensively studied According to Forest et al (6–9) plasma testosterone levels are elevated at birth in both sexes and diminish during the first week of life Then in males only they rise to a peak value at about three months of age before decreasing again to the prepubertal value which is reached at the age of six months Variations of plasma estradiol levels during this period were also reported In male infants after an initial decrease during the first week of life estradiol increases and reaches a peak at the end of the second month (2) In female infants occasional elevated estradiol levels are frequently found during the first two years of life (2 4)

The knowledge of these variations of plasma sex steroid concentrations suggested the possibility of related variations of TeBG binding capacity The aim of this study was to measure the serum TeBG binding capacity in infants of both sexes during the first year of life and to investigate the correlation of TeBG with plasma sex steroid levels

SUBJECTS AND METHODS

Serum TeBG binding capacity was measured in 1) cord blood samples from 4 boys and 4 girls 2) venous blood from 39 male infants aged 1 to 240 days 3) venous blood from 31 female infants aged 3 to 360 days

Plasma testosterone was measured in the venous blood

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DISCUSSION

Beischer et al (2) and Agboola (1) observed that maternal anaemia was associated with placental hypertrophy Similar observations at high altitudes by Kruger & Arias Stella (6) suggested that placental hypoxia was possibly responsible for this hypertrophy Surprisingly the results of the present study showed that the placental weight was significantly low in the mothers with severe anaemia The foetal birthweight and the placental volume were also significantly reduced in the severely anaemic mothers The high placental coefficient found in these mothers suggested that the weight of the newborn infant suffered more than the weight of the placenta in maternal anaemia

The reduced number of cotyledons in the placentae of mothers with severe anaemia indicated a lesser degree of septation and probably a greater proportion of functioning parenchyma (6) This could be an adaptation to a physiological stress resulting in an improvement of placental function and foetal well being

The present study showed a direct relation between the maternal and cord blood haemoglobin levels The mean cord blood haemoglobin was found to be markedly reduced in the mothers with moderate to severe anaemia These observations are in accordance with those reported recently by Nhonoli et al (8)

Although the foetus is an efficient parasite it is not clear whether the foetus takes the optimum amounts of iron or it takes amounts proportional to the levels available in the mother in pregnancy anaemia The recent work of Nhonoli et al (8) supports the latter possibility The linear relationships of the cord serum iron and transferrin saturation with the maternal haemoglobin found in this study further strengthen this view The low cord serum and placental iron levels in the severely anaemic mothers could be responsible for the poor iron stores in infants born to these mothers (3)

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The knowledge of these variations of plasma sex steroid concentrations suggested the possibility of related variations of TeBG binding capacity The aim of this study was to measure the serum TeBG binding capacity in infants of both sexes during the first year of life and to investigate the correlation of TeBG with plasma sex steroid levels

SUBJECTS AND METHODS

Serum TeBG binding capacity was measured in 1) cord blood samples from 4 boys and 4 girls 2) venous blood from 39 male infants aged 1 to 340 days 3) venous blood from 31 female infants aged 3 to 360 days

Plasma testosterone was measured in the venous blood

Supported by D G R S T Contract no 75 7 00 9

Table 1 *Individual values for serum TeBG plasma testosterone and estrogens in boys during the first year of life*

Age (days)	Testo-sterone (ng/ml)	TeBG (μg per 100 ml)	E1 (pg/ml)	E2 (pg/ml)
1	2 70	1 96		
2	0 90	2 18		
3	0 80	1 35		
3	1 00	1 93		
3	0 50	1 76		
4	0 30	3 10		
4	0 25	1 16		
5	0 40	3 43		
5	0 20	3 97		
5	0 60	1 61		
7	0 20	2 00		
8	0 20	2 58		
8	0 60	2 43		
11	0 30	4 25		
11	0 22	4 66		
12	0 20	1 58		
18	2 40	6 40		
21	0 80	2 42		
24	2 60	5 20	<10	11
26	1 90	7 00	23	15
44	3 20	13 11	25	34
47	4 50	6 74		
51	3 45	11 80		
53	7 40	9 30	19	26
57	1 50	6 03		
66	2 50	8 02	18	29
72	3 30	6 90		
75	4 35	7 31		
90	3 40	14 50	14	32
106	0 65	5 81	<10	10
122	1 50	4 69		
170	0 15	4 61		
180	0 05	3 16	10	20
184	0 28	4 00		
190	0 05	1 50		
209	0 12	4 36		
219	0 15	6 01	18	22
240	0 05	3 00	14	21
240	0 05	5 60	<10	14

of the boys and plasma E1 and E2 in the venous blood of the girls In addition plasma E1 and E2 were also measured in the venous blood of 11 males

TeBG binding capacity of serum for dihydrotestosterone (DHT) was measured by the steady state polyacrylamide gel method described by Ritzen et al (11) 30 μl of serum were incubated for 120 min at 0 C with ^{3}H DHT (0 064 nM in 40 μl) after dilution 1 16 in 10 mM tris HCl buffer containing 1 0 mM EDTA and 1 mM mercaptoethanol pH 7 4 at 25 with the addition of 10% glycerol v/v (TEMG) 100 μl of this solution were used for electrophoresis (2 mA 90 min) in polyacrylamide gel (acrylamide 8% bisacrylamide 2% ^{3}H DHT 16 nM/l) Then the gel was sliced and counted in Instagel (Packard Cy) At saturation the TeBG binding capacity of the serum expressed as μg/100 ml was calculated from the peak of radioactivity The Kd value used was 10^{-9} M In these conditions TeBG binding capacity (mean ± S D) was 6 23±1 72 in prepubertal boys 6 71±1 93 in prepubertal girls 1 79±0 75 in pubertal boys and 5 34±0 9[illegible] in pubertal girls

Plasma testosterone was measured by radioimmunoassay (10) Plasma was extracted with ether and purified on a sephadex LH 20 microcolumn Specificity was checked by combined gas liquid chromatography and mass spectrometry DHT was the only significantly interfering steroid the percentage of cross reaction being 49% As the mean plasma level of boys during the first year of life is less than 0 05 ng/ml this cross reaction lead to overestimation of plasma testosterone levels by less than 0 02 ng/ml Sensitivity was 0 012 ng/ml the intra assay variation was 7 1% and the interassay variation 16% at the 0 10 ng/ml level In this laboratory normal plasma testosterone values are 0 14±S E M 0 016 ng/ml in prepubertal boys 5 32±S E M 0 23 ng/ml in adult males

Plasma estrone (E1) and estradiol (E2) were radioimmunologically assayed according to Castanier & Scholler (3) Rabbit antiserum was obtained using estradiol and estrone 6-(O carboxy) Methyloxime BSA as antigens The bound and free fractions were separated using toluene scintillant (3 10) The detection limit was 10 pg for E1 and 6 pg for E2 The intra assay variation was 12% for E1 at the 31 pg/ml level and 9 1% for E2 at the 27 pg/ml level By this method the mean estrogen values (mean ±S D) in prepubertal boys were 10±0 9 pg/ml for E1 and 8 16±0 8 pg/ml for E2 and in prepubertal girls 10±1 8 pg/ml for E1 and 7 5±1 5 pg/ml for E2 Linear and polynomial regression curves of TeBG and testosterone have been calculated by the least squares method using a computorized programme (Hewlett Packard)

RESULTS

In cord blood TeBG values ranged between 0 80 and 1 62 μg/100 ml with a mean value of 1 27±S D 0 3 μg/100 ml No sex difference was observed

Individual values of TeBG plasma testosterone E1 and E2 in males are given in Table 1 and Fig 1 A highly significant correlation ($p<0$ 001) was found between TeBG binding capacity and age during the first three months of life (y=2 12 (95% confidence limits 0 09 to 3 25)+0 11 (95% confidence limits 0 08 to 0 14) x r=0 827 (0 653 to 0 920)) TeBG binding capacity was also correlated with plasma testosterone (y=3 01 (1 98 to 3 04)+1 31 (0 83 to 1 79) x r=0 671 (0 440 to 0 818) $p<0$ 005) and with plasma E2 levels (y=7 05 (2 85 to 12 2)+0 33 (0 10 to 0 56) x r=0 734 (0 121 to 0 942) $p<0$ 01) (Figs 2 and 3)

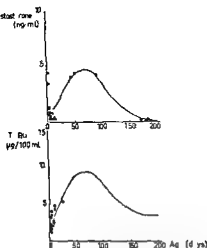


Fig 1 Variations of plasma testosterone and serum TeBG binding capacity in boys during the first year of life

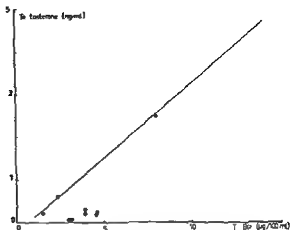


Fig 2 Correlation between serum TeBG binding capacity and plasma testosterone levels in boys during the first year of life

Individual values of TeBG and plasma E1 and E2 in females are given in Table 2 No correlation was found between individual values of TeBG and age or E1 and E2

DISCUSSION

Since the first study of TeBG in human pregnancy by Rivarola et al (12) it has been established that the TeBG binding capacity of serum is high in women during pregnancy and low in the fetus (5 13) The present study confirms that in both sexes TeBG binding capacity is very low in both cord blood and in peripheral blood from newborns Since estrogen levels in cord blood are very high (2) the concomitant low values of TeBG binding capacities suggest that the fetal production of TeBG is independent of estrogen levels The inhibiting role of the antenatal fetal adrenal secretion has already been discussed (1)

After the first week of life the variations in the serum TeBG binding capacity appear to be different in males and females In males a progressive increase of the TeBG binding capacity is observed from birth up to three months of age (Fig 1) Moreover a highly significant correlation between age and TeBG values exists during this period After three months of age the binding capacity decreases to reach the prepubertal level at the age of six months This diphasic pattern is parallel to the variations of plasma testosterone first reported by Forest et al (6–9) and confirmed in the male infants studied here (Fig 1) In prepubertal and adult subjects testosterone reduces TeBG binding capacity (13) Thus the fact that during the first three months of life TeBG is positively

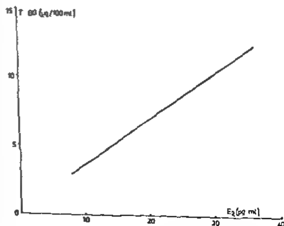


Fig 3 Correlation between serum TeBG binding capacity and plasma estradiol levels in boys during the first year of life

Table 2 *Individual values for serum TeBG and plasma estrogens in girls during the first year of life*

Age (days)	TeBG (µg per 100 ml)	E1 (pg/ml)	E2 (pg/ml)
3	3 15	20	28
3	1 19	26	42
3	3 15	–	–
5	2 80	28	17
5	1 17	23	14
5	3 63	30	26
6	3 41	25	28
6	3 44	–	–
7	2 97	–	–
8	3 05	18	17
10	6 37	–	–
20	7 28	10	9
30	12 27	28	45
40	5 91	18	8
60	13 07	24	35
76	3 00	10	6
90	2 00	19	30
91	4 72	22	45
93	7 45	24	39
150	3 57	–	–
153	14 00	12	10
180	12 6	16	23
190	14 5	–	–
255	6 96	13	21
320	6 38	20	23
330	6 45	15	54
360	3 19	13	6

correlated with plasma testosterone levels appears paradoxical This suggests a delay in the negative control of TeBG by testosterone the inhibiting effect occurring only after the age of three months It may also suggest that the increase of testosterone observed in boys in the first months of life is a passive phenomenon secondary to an increase in the serum level of TeBG A direct measurement of free testosterone levels in this age period may definitely settle this point

Estrogens in adolescent and adult subjects of both sexes increase the TeBG binding capacity (13) Bidlingmaier et al have shown that E2 levels reach a peak at three months of age in male infants (2) Thus the increase of TeBG binding capacity demonstrated in male infants during the first three months of life could be related to the increase of plasma estradiol levels during the same period In fact a positive correlation was demonstrated in the present study between these two parameters (Fig 3)

In female infants the variations of TeBG binding capacity are different from those observed in males (Table 2) Individual levels during the first year of life appear to be extremely variable from one child to another and do not correlate with age As variable levels of plasma estradiol have also been reported in girls during the first two years of life (2 4) it seemed logical to speculate that there is a relationship between TeBG and estradiol values However the present study failed to demonstrate such a correlation Marked instability of plasma E2 levels in infant girls during this period may explain this apparent discrepancy

In conclusion the present study demonstrates variations in the serum TeBG binding capacity during the first year of life which are different in the two sexes The positive action of estrogens on TeBG binding capacity probably exists in the first month while the negative effect of testosterone seems to be delayed until after three months of age

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RELATIONSHIP BETWEEN HAEMOGLOBIN AND SERUM TESTOSTERONE IN NORMAL CHILDREN AND ADOLESCENTS AND IN BOYS WITH DELAYED PUBERTY

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From the Children s Hospital Fuglebakken University Department of Paediatrics Medi Lab A/S Copenhagen the Department of Clinical Chemistry Glostrup Hospital Glostrup and the Division of Endocrinology Department of Internal Medicine Hvidovre Hospital University Hospital of Copenhagen Hvidovre Denmark

ABSTRACT Krabbe S Christensen T Worm J Christiansen C and Transbøl I (Children s Hospital on Fuglebakken Medi/Lab A/S Copenhagen Department of Clinical Chemistry Glostrup Hospital Glostrup and Division of Endocrinology Department of Internal Medicine Hvidovre Hospital Hvidovre Denmark) Relationship between haemoglobin and serum testosterone in normal children and adolescents and in boys with delayed puberty Acta Paediatr Scand 67 655 1978 —The concentrations of haemoglobin and of serum testosterone were measured in 215 normal children and adolescents aged 7–20 years and in 8 boys with constitutional delayed puberty From the age of 14 years onward haemoglobin and testosterone rose in normal boys and differed significantly from the stable levels observed in prepubertal children and pubertal girls In the entire series of normal boys (n=118 age 7–20 years) concentrations of haemoglobin and testosterone were found to be closely correlated (r=0 73 p<0 001) These results provide further evidence for a major role of testosterone in the control of erythropoiesis Therefore this correlation suggests the use of serum testosterone determination for the proper selection of haemoglobin reference ranges in boys The respective reference ranges of haemoglobin corresponding to testosterone levels ■ 0 and 30 nmol/l were 120 5–148 5 and 143 5–171 5 g/l (95% confidence limits) Boys with delayed puberty were found to have significantly reduced median values of haemoglobin and testosterone for their chronological age and 6 of the 8 boys investigated were truly anaemic on this background Nevertheless their haemoglobin concentration did appear appropriate as judged from their testosterone levels This observation supports the idea that the selection of the relevant reference range for haemoglobin in boys should depend on the state of physical development as expressed by serum testosterone

KEY WORDS Haemoglobin testosterone puberty

The haemoglobin concentration remains nearly constant in the female sex from childhood through senescence while that of boys begins to exceed female levels at the onset of puberty (4) The discrepancy between the haemoglobin levels of boys and girls becomes apparent after 13 to 14 years of age reaches a maximum in the twenties and declines gradually towards the seventies (4) This pattern suggests the use of age and sex matched reference groups in the diagnosis of anaemia On the other hand chronological age may be ■ bad indicator of biological development during puberty since a wide range of growth and sexual maturation is seen in individuals of the same age in this period (7) It has been shown that the concentration of testosterone correlates well with the pubertal stage (6) which is a better expression of the physical development during adolescence than is the chronological age Recalling the stimulatory action of androgenic hormones upon erythropoiesis (5 10) and the similar time courses of plasma testosterone (6) and haemoglobin levels (above) one would expect a high correlation between the concentrations of haemoglobin and testosterone

To test this hypothesis we have measured

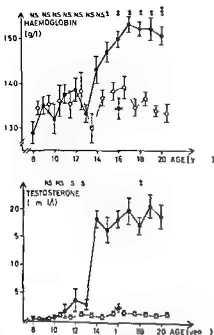


Fig 1 The concentrations of haemoglobin (above) and serum testosterone (below) as functions of age (mean ±1 SEM) in normal males (●) normal females (O) and in males with delayed puberty (■) Significance of difference between mean values of normal males and females N S = not significant + = $p<0.05$ ++ = $p<0.01$ +++ = $p<0.001$

RESULTS

Normal children and adolescents

The results are presented in Figs 1 and 2 In girls haemoglobin and serum testosterone concentrations remain at the same levels throughout all age groups (Fig 1) Comparable levels of both variables are observed in boys below the age of 14 years Thereafter the haemoglobin and testosterone concentrations rise concomitantly rapidly attaining levels which differ significantly from those of pre pubertal children and of pubertal girls (Fig 1) Correlation of all values of haemoglobin and testosterone from the entire group of boys reveals a highly significant relationship (Fig 2 $r=0.73$ $p<0.001$) Inspection of Fig 2 discloses that the appropriate normal ranges of haemoglobin concentration at serum testosterone levels of 0 and 30 nmol/l are 120 5–148 5 and 143 5–171 5 g/l respectively (95% confidence limits)

Boys with delayed puberty

The median values of haemoglobin and testosterone concentrations are 134 g/l (range 128–147) and 2 4 nmol/l (range 1 7–7 6) respectively Both values are significantly lower than those of age matched normal boys (Fig 1 $p<0.001$) It is seen from Fig 2 that the separate sets of observations are within the 95% confidence limits of the normal haemoglobin/testosterone relationship Comparing the individual values of haemoglobin in this group with those of normal boys (Fig 1) reveals that six of the boys with delayed puberty were anaemic for their chronological age (2 S D below mean)

DISCUSSION

Androgenic hormones have been used as a forceful stimulator of haemoglobin production in a variety of clinical conditions (5 10) Besides this the previous observation of anaemia in primary and secondary hypogonadism in men and its correction by androgens (5) together with the synchronous time courses followed by haemoglobin (4) and plasma testo-

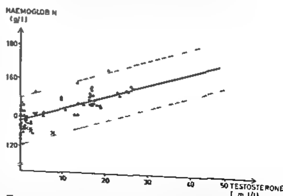


Fig 2 The correlation between haemoglobin and serum testosterone concentrations in 118 males aged 7–20 years ($r=0.73$ $p<0.001$) The corresponding values of haemoglobin and serum testosterone concentrations in 8 boys with delayed puberty aged 14–17 years (×) The 95% confidence limits are given by the dashed lines

Table 1 *Distribution of height weight sex and age in 215 normal children and adolescents*

	Boys					Girls				
		Height (cm)		Weight (kg)			Height (cm)		Weight (kg)	
Age (y)	No of subjects	Mean	S D	Mean	S D	No of subjects	Mean	S D	Mean	S D
7–8	3	133 3	4 7	27 7	3 1	7	130 8	7 4	28 0	5 7
9	7	139 4	8 9	31 8	4 8	4	145 8	12 7	35 6	8 0
10	4	147 9	7 1	39 4	7 0	3	145 8	3 8	37 7	9 9
11	11	149 4	6 2	38 8	7 0	6	143 8	4 1	35 6	5 8
12	8	145 7	2 0	37 0	5 9	3	157 2	5 4	47 0	8 0
13	13	148 8	2 5	34 1	5 9	3	166 9	8 4	52 2	7 7
14	8	172 2	8 9	55 3	10 3	11	163 6	7 2	49 8	6 9
15	11	173 9	10 9	65 2	15 5	12	166 7	6 3	59 4	10 7
16	8	175 6	10 5	60 0	10 6	8	163 6	6 1	57 3	13 5
17	14	182 2	7 2	68 6	8 0	9	166 8	4 1	57 3	9 5
18	10	179 4	5 4	68 1	6 5	11	165 9	7 1	57 6	11 7
19	11	176 8	5 3	[illegible] 4	7 8	10	168 [illegible]	5 5	56 7	6 1
20	10	178 0	5 0	70 6	5 9	10	166 2	4 4	56 9	5 7

these variables in groups of normal boys and girls and of boys with delayed pubertal development

PATIENTS AND METHODS

Normal children and adolescents

A total of 215 normal children and adolescents between 7 and 20 years of age participated in the study They were randomly selected among the pupils of two schools in a suburban area Pupils below 18 years of age obtained a written consent from their parents All were in good health without symptoms of gastrointestinal or renal diseases No one took contraceptive pills or any other type of drugs Data concerning sex age weight and height are given in Table 1

Boys with delayed puberty

Eight boys referred to the clinic for delayed growth and/or pubertal development were selected for the study (Table 2) The diagnosis was constitutional or familial delayed puberty (12) without signs of other endocrine diseases Based on data on pubertal changes in normal boys (7) the retardation in pubertal development was 2 standard deviations (S D) or more in six boys and between 1 and 2 S D in two boys Testicular size was within the pubertal range in all subjects

METHODS

Blood samples were drawn between 8 and 12 a m in normal children and during early afternoon in the boys with delayed puberty None of the subjects were fasting All samples were drawn from cubital veins Height and weight were measured with the subjects dressed but shoeless

Serum testosterone was measured by radioimmunoassay including a modification of thin layer chromatography (8) Statistical methods The Wilcoxon test and regression analysis were used

Table 2 *Clinical data and the concentrations of haemoglobin and serum testosterone in eight boys with delayed puberty*

No	Age (y)	Height (cm)	Bone age (y)	Pubic hair[b]	Haemoglobin concentration (g/l)	Serum testosterone concentration (nmol/l)
1	14 7/12	148	12	I	137	1 0
2	14 11/12	151	13	II	147	2 6
3	15 7/12	150	13	I	129	2 5
4	15 8/12	157	14	II	130	1 1
5	15 10/12	157	13	II	130	2 3
6	16 9/12	162	13	III	128	7 6
7	16 10/12	154	13	I	134	1 3
8	17 0/12	160	14	II	139	3 6

According to Greulich W W & Pyle S I (3)
[b] According to Tanner J M (11)

Acta Pædiatr Scand 67 659–663 1978

PLASMA FREE AMINO ACID CONCENTRATIONS OF BREAST FED INFANTS

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ABSTRACT Lindblad B S Alfven G and Zetterstrom [redacted] (Department of Paediatrics of Karolinska Institutet at St Goran s Children s Hospital Stockholm Sweden) Plasma free amino acid concentrations of breast fed infants Acta Paediatr Scand 67 659, 1978.—Photometric determination of alpha amino nitrogen in peripheral venous plasma and urine from 20 healthy full term infants 1–5 months of age showing normal growth and development during an uncomplicated lactation revealed lower plasma levels than what has been found in adults or 3 7±1 1 mg/100 ml and a urinary excretion of 41±14 mg/24 hours Ion exchange chromatography of deproteinized peripheral venous plasma showed low valine concentrations an increased glycine/valine ratio and high cystine and very high taurine levels when compared to the levels of healthy American infants of comparable ages fed 3–3 5 g/kg of cow milk protein The findings indicate that a formula based on cow milk protein should optimally contain only 1 0–1 2 g protein/100 ml provided that it is humanized not only with regard to the lactalbumin/casein ratio but also to the cystine and taurine content The pattern of the plasma concentrations of free amino acids reported in the present investigation may be used as a normal reference for breast fed infants

KEY WORDS Amino acids plasma breast feeding human milk cow milk formula valine cystine taurine protein requirement

The free amino acid concentrations of plasma as measured by ion exchange chromatography show little inter individual variation if certain precautions are met with in the sampling storage and analytical procedures (30) However the plasma levels are dependent on age and sex (2) and on the protein intake (1 16 31) There are four investigations known to the authors where the plasma levels of free amino acids during childhood have been correlated to age and to dietary intake two in premature infants (27 28) one in full term infants during formula feeding (31) and one in children 7–13 years of age (17)

In view of the increasing need for amino acid analysis of plasma during screening diagnosis and follow up of inborn errors of amino acid metabolism (19) during the monitoring of total parenteral nutrition (22) and during the early diagnosis of nutritional inadequacy (23) there is obviously a need to establish the levels of breast fed full term infants as opposed to those of infants fed cow milk formula with a considerably higher protein intake

The present investigation therefore aims at determining the post alimentary free amino acid levels of deproteinized peripheral blood plasma in normal human infants as represented by healthy full term infants showing normal growth and development during an uncomplicated lactation

MATERIAL AND METHODS

Twenty mothers of a mean age of 24 years who were selling their excess breast milk to the Stockholm milk distribution centre were hospitalized with their healthy 1 to 5 month old (mean 80-day old) infants The mothers total milk volumes were 1244±381 ml/24 hours according to test weighing and mechanical pumping of excess milk at home before admission The infants 10 girls and 10 boys were all full term and showed a normal growth from normal birth weights and heights and were healthy with a normal development They had all been fed ad libitum from the breast

Supported by a grant from the Swedish Medical Research Council (no 2583)

sterone concentrations (8 9) from childhood through senescence strongly suggest a dependence of haemoglobin production upon testosterone secretion in males

Our results confirm that great increases in the concentrations of haemoglobin and testosterone occur in normal males during adolescence Daniel (2) found that haemotocrit values and sexual maturity ratings were interrelated in normal boys This relationship is substantiated by the high degree of correlation between haemoglobin and testosterone observed in the present study These findings suggest that testosterone governs the level around which haemoglobin production is feed back regulated The reality of this dependence is supported by our observations in boys with delayed puberty They had anaemia and hypogonadism for their chronological age but appropriate haemoglobin concentrations when their low levels of testosterone were taken into consideration (Fig 2)

The demand for appropriate normal standards can generally be met by application of age and sex matched normal controls However in periods of rapid human development where chronological age and physical development may diverge substantially this approach is of restricted usefulness The importance of using appropriate standards especially during adolescence has been stressed by others (1 2)

If the variable in question depends on the state of physical development rather than on chronological age as it appears to be the case with haemoglobin serum testosterone may turn out to be the basic criterion from which the appropriate reference range can be deduced

ACKNOWLEDGEMENT

The study was supported by grants from Kong Christian X s foundation and the Foundation of Grosserer A V Lykfeldt og Hustru

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Table 2 *Plasma valine concentrations during childhood*

Results which can be regarded as normal plasma levels for age are in italics

Investigation (reference)	Result µmol/l M±2 S E
Newborn infants before feeding (?4)	*122±10*
Premature babies 1-? weeks old (?8)	
Human milk	139±34
Formula 1 8 g/100 ml	?19±36
Formula ? 3 g/100 ml	274±44
Premature babies less than 8 weeks old (?7)	
170 ml human milk/kg (800 µmol VAL/kg)	1?0
Formula 1 5 g/100 ml (1 300 µmol VAL/kg)	180
Formula 3 g/100 ml (? 500 µmol VAL/kg)	?80
1–5 months of age human milk (present investigation)	*113±2?*
Ninth day of life formula feeding (16)	247±38
1–3 months of age formula feeding (31)	
1 3 g protein/kg	91
1 5 g protein/kg	111
1 7 g protein/kg	167
3–3 5 g protein/kg	194±18
9 g protein/kg	59
4 days–1 year of age (23)	*153± 6*
4 days–1? years of age (?5)	*171± 4*
4 months–*2.5 years* of age (16)	222± 1
1–5 years of age Kwashiorkor (18)	66± 6
7–13 years of age (17)	
0 ?1 g protein/kg	194±55
1 9 g protein/kg	*212±50*
3 1 g protein/kg	457±3?
6–18 years of age (?)	*223± 7*
Adults (?)	*252± 8*

slightly lower than that reported for adults (4) Moreover the individual amino acid levels of plasma show considerably lower branch chained (valine leucine isoleucine) amino acid levels in comparison with those of older children and adults (2 5 17 25) The dependency on age of the valine plasma levels on the one hand and on dietary protein intake on the other is quite evident from the review of the literature summarized in Table 2

When the present results are compared with the plasma levels of free amino acids of control American infants of 1–3 months of age being fed cow milk based formula in amounts 3–3 5 g protein/kg in an investigation demonstrating the effect of overfeeding (9 g protein/kg) on the plasma aminogram (31) it is clear that even the controls show the high valine levels and decreased glycine/valine quotient characteristic of protein overnutrition (23 31) This difference between normal breastfed Swedish young infants consuming 0 82 g protein/100 ml (21) and normal cow milk formula fed American young infants consuming 2–2 3 g protein/100 ml (Fig 2) could be of importance as there are indications that the branch chained amino acid and tyrosine levels of plasma in particular influence insulin metabolism The branch chained amino acid levels of plasma seem to be especially sensitive to exogenous and endogenous insulin (6 9 10 26 35) Valine leucine isoleucine phenylalanine and tyrosine levels (compare Fig 2) were significantly elevated in obese subjects (9) and inversely correlated with immunoreactive insulin levels The branch chained amino acid levels are also significantly elevated in patients with diabetic acidosis (11) It has therefore been suggested (9) that leucine which apparently induces insulin release by a different mechanism from that of glucose and arginine (8) acts as a physiological insulin feed back regulator

In Fig 3 the plasma valine level of small infants during breast feeding is compared with that of newborn infants before feeding (7 20 24) and the plasma levels of infants on different amounts of cow milk protein feeding (31) It is evident from this figure that in order to reach a plasma valine level equivalent to that of breast fed infants the cow milk based formula should be consumed in amounts of 1 5 g/kg This is considerably lower than what is customary but is comparable to the requirement for protein of normal full term infants of no greater than 1 72 g/100 kcal (1 15 g/100 ml) found by Fomon et al (12)

The low cystine and taurine levels in formula fed infants are consistent with the low cystine content of casein and non humanized

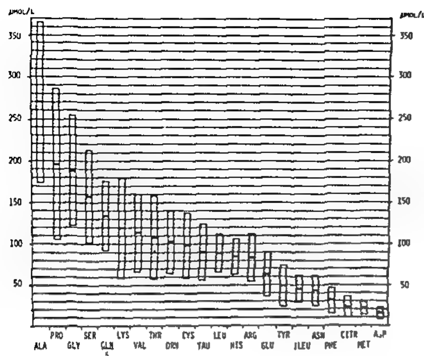


Fig 1 The free amino acid levels of peripheral venous blood plasma of 1 to 5 month old breast fed human infants Mean ± S D are given

Blood was collected from the cubital vein in a heparinized tube before the early morning feed The blood sample was centrifuged immediately the plasma was deproteinized with crystalline sulphosalicylic acid 50 mg/ml and the supernatant stored in −72 C until analyzed for alpha amino nitrogen (29) and for individual free amino acids on a Bio Cal 200 (München) automatic amino acid analyser according to the method of Spackman et al (32) A lithium buffer system (3) was used allowing for the separation of serine glutamine and asparagine Tryptophan is lost during deproteinization and analysis as it is partly bound to albumin and is not determined by this method Cysteine was oxidized to cystine by allowing the sample to stand in room temperature at pH 7 for 4 hours The methionine sulphone peak was added to the one representing methionine during the estimation of the methionine concentration Reproducibility was mean 4 9±0 5 (SE)% for the 23 parameters determined by ion exchange chromatography ranging from 1 2% (glutamine) to 9 4% (cystine)

Urine was collected during a 24 hour period The urine was kept in a refrigerator during collection and stored in −72 C until analyzed for alpha amino nitrogen content (29)

RESULTS

The alpha amino nitrogen concentration of plasma during uncomplicated lactation was found to be 3 7±1 1 ($n=11$) mg/100 ml and the urinary excretion 41±14 ($n=20$) mg/24 hours

The individual free amino acid levels and the urea concentrations are given in Table 1 The results are also given in the form of an aminogram in Fig 1 which can serve as a reference chart on which to plot the results of plasma amino acid analyses during infancy

DISCUSSION

The alpha amino nitrogen concentration of plasma found in the present investigation is

Table 1 *The plasma free amino acid and urea concentrations of breast fed infants 1-5 months of age*

Amino acid	μmol/l	S D	n	S E
Taurine	90	34	19	8
Aspartic acid	15	7	19	2
Threonine	108	50	19	11
Serine	156	56	19	13
Glutamine	663	208	19	48
Asparagine	43	*18*	*18*	4
Glutamic acid	63	27	19	6
Citrulline	23	13	6	5
Proline	195	90	19	21
Glycine	187	66	19	15
Alanine	269	96	19	22
1/2 Cystine	98	39	19	9
Valine	113	47	18	11
Methionine	21	8	18	2
Isoleucine	45	16	19	4
Leucine	89	23	19	5
Tyrosine	49	25	19	6
Phenylalanine	31	15	17	4
Ornithine	102	38	16	10
Lysine	119	61	19	14
Histidine	84	22	19	5
Arginine	83	29	16	7
Urea	2 984	1 613	19	375

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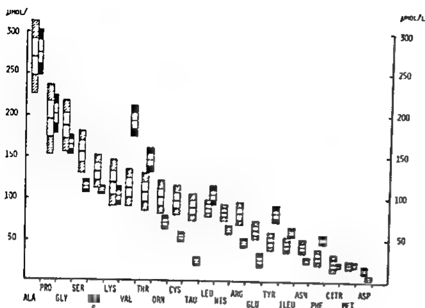


Fig 2 Peripheral venous plasma free amino acid concentration of 1 to 5 month-old human infants Partly striped bars indicate breast fed infants (mean ±2 S E $n=19$) partly filled bars cow milk formula fed infants 3–3 5 g protein/kg (mean ±2 S E $n=29$ ref 31) The glutamine concentrations have been divided by 5 before being included in the diagram The levels during formula feeding of glutamine glutamic acid and aspartic acid are of infants 1–2 years of age (ref 33 using a lithium buffer system identical to the one of the present investigation)

cow milk formula and the low amounts of taurine in cow milk as compared to human milk (14, 15) This finding indicates a suboptimal supply of cystine and taurine to cow milk formula fed infants which could be important as cystine is an essential amino acid at least for prematurely born human infants (13) Besides cystine is a precursor to taurine which seems to be essential and to play a major role in brain development (14 34)

The hypermethioninemia of excessive protein intake of infants accidentally discovered during screening for inborn errors of amino acid metabolism in the U S (19) is not seen during 3–3 5 g/kg cow milk protein feeding when compared with breast feeding (Fig 2)

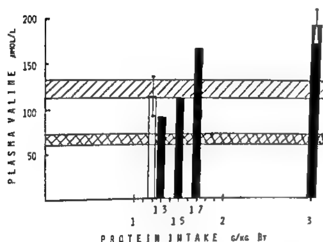


Fig 3 Peripheral venous plasma valine levels during early infancy The open bar indicates the result of the present investigation (breast fed infants 1–5 months of age) the filled bars those of Snyderman et al (31) during different forms of cow milk protein feeding 1 3 1 5 1 7 and 3 g protein/kg in infants 1–3 months of age The hatched area represents mean ±2 S E of newborn infants before the start of feeding procedures during the first day of life (24) The cross hatched area represents mean ±2 S E of infants 1–5 years of age with the clinical syndrome of kwashiorkor (protein deficiency) from 5 different developing countries (18)

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Acta Pædiatr Scand 67 665–666 1978

LETTER TO THE EDITOR

Recent Increase in Breast Feeding

Sir

Recently we have completed a survey of infant feeding in the municipality of Copenhagen 1938–72 which we hope to publish in near future (1) We found certain indications that nursing habits were changing around 1972 Due to administrative changes this was unfortunately the last year for which the infant health visitors files were accessible in the form fit for our survey Breast feeding had been on a decline since World War II but after 1968 it seemed to regain popularity among the young generation of mothers who though often unmarried were having their firstborn babies during those years In particular young unmarried mothers showed a tendency to continue breast feeding for several months once they got started Furthermore 1970 saw a flattening of the all Denmark sales curve for powdered milk formulas although this had been rising exponentially for two decades (by no less than 25% per year!) After a peak value of 7 23 kg milk powder sold per liveborn in 1971 it has settled down to 90–95% of that value

Following this lead we decided to study a representative sample of health visitors records for children born 1973–1976 We extracted two readily accessible items of information viz duration of breast feeding and whether or not the infant was born in wedlock The startling results are shown in Fig 1 In view of the agreement between the 1973 sample and the exhaustive data for 1972 (1) the subsequent rise can hardly be an artifact Not only is the average duration of breast feeding back at the level of the early sixties but infants born out of wedlock who now constitute no less than 1/3 of all births are now breast fed longer than those born in wedlock

In fact we see a return to the level which prevailed before 1951 but in view of societal changes this is perhaps not a relevant comparison

Several factors may have contributed to these trends including new attitudes towards marriage and family life particularly amongst those who inhabit the city to day It should not be forgotten that many nuclear families have moved to the suburban areas not covered by our data Again unemployment may have contributed to prolonged nursing No doubt

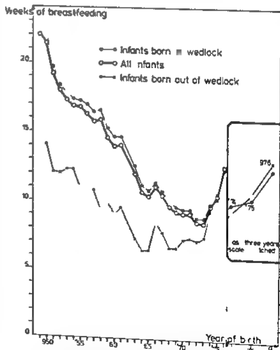


Fig 1 Average duration of breast feeding in the Municipality of Copenhagen 1949–76 according to infant health visitors records Data for 1949–72 based on exhaustive statistics data for 1973–76 based on a sample Each point represents between 243 and 7 992 children the associated standard error is always less than 0 4 week

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CASE REPORT

DISSEMINATED HERPES SIMPLEX VIRUS INFECTION IN ATAXIA TELANGIECTASIA

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ABSTRACT Ben Zvi A Soffer D and Yatziv S (Departments of Paediatrics and Pathology Hadassah University Hospital Jerusalem Israel) Disseminated herpes simplex virus infection in ataxia telangiectasia Acta Paediatr Scand 67 667 1978.—The clinical and pathological features are described in a child with ataxia telangiectasia complicated by fatal disseminated herpes simplex virus infection Herpes simplex virus was isolated from the patient s blood and the histopathological findings in the skin liver and adrenals were consistent with herpes simplex virus infection The patient had a combined immune deficiency state as a part of the ataxia telangiectasia syndrome She had impaired cellular immune response to herpes simplex virus and developed no antibodies against the virus To our knowledge this is the first fatal case of disseminated herpes simplex virus infection in ataxia telangiectasia

KEY WORDS Ataxia telangiectasia herpes simplex virus

Ataxia telangiectasia (A T) is a rare familial disorder characterized by the appearance in childhood of cerebellar ataxia ocular and cutaneous telangiectasia and frequent severe respiratory infections (3 12) Another important feature of the disease is the occurrence of an immune deficiency state characterized by defective humoral immunity (6 19 25) and frequently abnormal cellular immunity as well (4 7 13 19) In spite of the immune deficiency and the frequent occurrence of respiratory infections no disseminated herpes simplex infection has been reported in A T patients to the best of our knowledge (12)

We wish to present the clinical and pathological findings in a typical case of A T complicated by a fatal disseminated herpes simplex virus (HSV) infection associated with disseminated intravascular coagulopathy (DIC)

CASE REPORT

A 7 year-old girl was admitted to the department of Paediatrics with repeated respiratory tract infections and involuntary movements She was the fourth child to consanguinous parents Two of her brothers were healthy but a third died at the age of 11 years from a disease resembling A T The patient developed uneventfully until the age of eighteen months At this age it was noted that her gait was unsteady Since the age of 7½ years repeated frequent respiratory tract infections occurred and at the age of one year a red discoloration was already noted in her eyes On admission she was pale and cyanotic Telangiectases were seen in the conjuctivae fine moist rales were heard over both lungs and her temperature was 39 C Jaundice petechial skin haemorrhages and generalized herpetic skin eruption were observed as well as numerous buccal vesicles The liver was palpated 3 cm below the costal margin Neurological examination revealed mask face and a cerebellar syndrome characterized by ataxia with broadbased gait dysarthria dysdiadochokinesis incoordination of limbs and a positive Romberg sign

Laboratory examinations showed hypochromic anaemia mild leukocytosis with granulocytosis severe electrolyte imbalance (BUN 14 mmol/l sodium 118 mmol/l potassium 3 1 mmol/l chloride [redacted] mmol/l uric acid 720 µmol/l) and severe liver function disturbance (total serum proteins 56 g/l albumin 15 g/l bilirubin 85 µmol/l alkaline phosphatase 710 SMA units SGOT 470 IU) Immunoelectrophoresis revealed 0 84 g/l IgM 12 5 g/l IgG and 0 72 g/l IgA Chest X rays showed bronchiectasis and pneumonia Herpes simplex virus was isolated from the throat from a skin lesion and from the blood Repeated blood cultures for bacteria and fungi were negative No

however the rapid increase is also due to the growing opinion that natural food—including breast—will serve us best

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1 Biering Sørensen F Hilden J & Biering Sørensen K Breast feeding in Copenhagen 1938–1977 Data for more than 360 000 infants To be published

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CASE REPORT

DISSEMINATED HERPES SIMPLEX VIRUS INFECTION IN ATAXIA TELANGIECTASIA

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ABSTRACT Ben Zvi A Soffer D and Yatziv S (Departments of Paediatrics and Pathology Hadassah University Hospital Jerusalem Israel) Disseminated herpes simplex virus infection in ataxia telangiectasia Acta Paediatr Scand 67 667 1978.—The clinical and pathological features are described in a child with ataxia telangiectasia complicated by fatal disseminated herpes simplex virus infection Herpes simplex virus was isolated from the patient s blood and the histopathological findings in the skin liver and adrenals were con sistent with herpes simplex virus infection The patient had a combined immune deficiency state as a part of the ataxia telangiectasia syndrome She had impaired cellular immune response to herpes simplex virus and developed no antibodies against the virus To our knowl edge this is the first fatal case of disseminated herpes simplex virus infection in ataxia telangiectasia

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as the inconstant findings of vascular malformation in the cerebellum (3 22)

The most unusual feature of our case was the intercurrent disease cuasing the patient s death—disseminated HSV infection Clinical laboratory and pathological findings supported this diagnosis and were indicative of generalized HSV infection with liver involvement (4 7 10 11) HSV was isolated from a skin lesion from the throat and from the blood The pathological picture of the skin lesions is pathognomonic for the herpes simplex varicella zoster group The findings of necrotizing hepatitis and adrenal necrosis are frequently observed in HSV infection (2 9 11 14 18 27) Widespread haemorrhages with DIC which were ■ prominent feature of the terminal disease are also common in disseminated HSV infection occurring in 40% of the cases (14) and the absence of fibrin microthrombi can be attributed to possible fibrinolytic activity (24)

Beside the newborn HSV can cause disseminated infection in cellular immunologically impaired persons (5 16 17 23) These include patients on steroid and immunosuppressive therapy (4 11 18) patients with haematologic malignancies (15) and those with Wiskott Aldrich syndrome (23) In addition a few cases of visceral HSV were reported in pregnant women (7 8 26) and in young children with severe malnutrition (kwashiorkor) (2) Humoral factors may have a role in conjunction with cellular immunity (16) Our patient had no humoral antibodies or cellular response to HSV which explains the disseminated and fatal infection

Although HSV has a widespread distribution (17) and unresponsiveness to viral antigens including HSV is common in A T (12) surprisingly no cases of disseminated HSV infections have been yet reported in this disease

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Fig 1 The thymus is almost completely replaced by fat tissue The remaining small amount of thymic parenchyma consists mainly of reticulated endothelial cells with only a very small number of lymphocytes No Hassall s corpuscles were found (Haematoxylin and eosin ×32)

antibodies to HSV were found in the serum during the three weeks after the appearance of the herpetic lesions Tests of cellular immunity showed no blastic transformation of lymphocytes with phytohaemaglutinin (PHA) with concanavallin A (Con A) or with herpes simplex virus Coagulation parameters were severely disturbed indicating disseminated intravascular coagulopathy (DIC) (fibrinogen 0 8 g/l platelets 50000/mm^3 clotting time 9 min clot retraction 19% prothrombin time 12% partial thromboplastin time 70 sec (control=40 sec) euglobulin lysis time started after one minute and thrombin time 17 sec (control=10 sec))

The diagnosis of disseminated HSV infection associated with DIC and ataxia telangiectasia was made and treatment with blood transfusions albumin gamma globulin fresh plasma heparin and ampicillin was initiated Some improvement in the coagulation tests was observed during treatment but the general condition of the child continued to deteriorate and she died on the eleventh day of hospitalization

Autopsy findings the main findings at autopsy were marked lymphoid tissue abnormalities involving thymus lymph tissue of the gut pulmonary infection in the form of chronic bronchitis bronchiectasis and pneumonia generalized HSV infection involving almost all the skin of the body and oral mucosa necrotizing hepatitis and massive haemorrhagic necrosis of the adrenals In addition there was hemangiomatosis of the cerebellar meningeal vessels

The thymus was small composed of small lobules separated from one another by fat tissue The lymph nodes also showed severe lymphocytic depletion in the cortex and paracortex The medullary sinuses were extremely dilated and filled with many plasma cells Similar lymphocytic depletion was found in the gut especially in the appendix where marked depletion was seen in the submucosa In the cerebellum a small vascular hamartoma was found located in the leptomeninges and extending into the cerebellar parenchyma The liver weighed 870 g Histologically there were widespread areas of confluent necrosis in a haphazard distribution with a minimal inflammatory response Both adrenals showed massive haemorrhagic necrosis with minimal inflammatory reaction No inclusion bodies were found

The skin lesions showed changes typical for HSV Along the base of the vesicles many multinucleated epithelial giant cells were seen as well as many cells with typical ground glass appearance A few intranuclear eosinophilic inclusions bodies were found (Fig 1)

DISCUSSION

The patient showed the classical clinical and pathological features of A T She suffered from a familial disease characterized by the occurrence in early childhood of cerebellar ataxia ocular telangiectasia recurrent respiratory tract infections and a combined immune deficiency state This combination of signs and symptoms is diagnostic for A T (3 12) The diagnosis was confirmed pathologically by the presence of characteristic changes in the thymus lymphatic tissue and cerebellum Thymic maldevelopment is an integral part of A T (12) The thymus resembled the foetal thymus prior to differentiation into a lymphoid organ a picture characteristic for A T (1 12 19 20 21) The generalized lymphocytic depletion in the intestine and lymph node is also a major component of A T (12 20 21) as well

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CASE REPORT

PARADOXICAL ENHANCEMENT OF TOLBUTAMIDE INDUCED INSULIN RELEASE BY DIAZOXIDE IN A PATIENT WITH ISLET CELL HYPERPLASIA

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From the Section of Endocrinology Department of Medicine Abraham Lincoln School of Medicine University of Illinois College of Medicine the Medical Service VA West Side Hospital Chicago Illinois and the Clinical Investigation Service Madigan Army Medical Center Tacoma Washington USA

ABSTRACT Schikman C H Chertow B S and Fariss B L (Section of Endocrinology Department of Medicine Abraham Lincoln School of Medicine University of Illinois College of Medicine and Medical Service VA West Side Hospital Chicago Illinois and Clinical Investigation Service Madigan Army Medical Center Tacoma Washington USA) Paradoxical enhancement of tolbutamide-induced insulin release by diazoxide in a patient with islet cell hyperplasia Acta Paediatr Scand 67 671 1978.—A case of islet cell hyperplasia in a ten year old black male with symptomatic fasting hypoglycemia was documented histopathologically Provocative studies with glucose tolbutamide glucagon and diazoxide were performed to test the insulin response of hyperplastic islets The islets responded to glucose glucagon and tolbutamide Diazoxide potentiated the tolbutamide induced insulin response and this effect of diazoxide was not blocked by propranolol In the diagnostic work up of islet cell hyperplasia dizoxide may paradoxically potentiate tolbutamide-induced insulin release a finding which may falsely suggest progression of the disease

KEY WORDS Islet cell hyperplasia tolbutamide diazoxide propranolol

Hyperplasia of the islets of Langerhans is a well established clinical and pathological entity (13 14 16 17) The present case which is documented histopathologically is of a child who had extensive provocative tests to elucidate basic mechanisms of insulin secretion from his hyperplastic islets In addition the phenomenon of diazoxide induced potentiation of insulin release recently described in animals and man is reported in our patient

CASE REPORT

O S was first seen at Madigan General Hospital (Case 7717) in 1970 at age 9 He was well until the winter of 1969 at which time he began to have difficulty concentrating and often stared into space His mother could avoid his staring episodes with feedings He had no access to [redacted] oholic beverages or hypoglycemic agents and he did not ingest excessive aspirin Physical examination revealed a healthy looking well developed 9 year old black male in no distress who was cooperative and alert His height was 140 cm weight 45 kg The remaining physical examination was within normal limits

A complete blood count urinalysis and chest and skull X rays were normal Fasting blood glucose levels on separate occasions were 2 2 2 4 1 9 1 9 and 1 1 mmol/l A Pitressin® stimulation test showed normal pituitary adrenal function and a glucagon stimulation test with a normal increase in plasma glucose ruled out an abnormality in glycogen storage A tolbutamide stimulation test was compatible with organic hyperinsulinism The diagnosis of a possible islet cell adenoma was made at that time and the patient started on diazoxide 100 mg twice daily

In June 1971 approximately [redacted] months after initiation of diazoxide another tolbutamide tolerance test showed severe hypoglycemia and hyperinsulinism Because of the suspicion of an islet cell tumor an abdominal and pancreatic exploration was performed on January 26 1972 A pancreatic tumor was not found and [redacted] partial pancreatectomy with only 50% resection of the body and tail was performed Microscopic review of multiple sections of the pancreas (Fig 1a and 1b) revealed many islets containing a few very large cells with large nuclei and rare islets that were substantially enlarged The large cells

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seemed to be mostly beta cells when evaluated with special stains A few foci of islet neoformation from ducts (nesidioblastosis) were present which have been described to occur in and around pancreatic endocrine tumors (7)

MATERIALS AND METHODS

Plasma glucose was measured on an autoanalyzer by the ferricyanide reducing method Insulin (normal fasting serum values <10 μU/ml) and glucagon (normal fasting levels of unextracted plasma 50–200 pg/ml using 30 K antiserum) were determined by radioimmunoassay (8 11) Informed consent for all studies was obtained from the mother of the patient During provocative tests a physician was present and symptomatic hypoglycemia was immediately treated [1]

Oral Glucose Tolerance Test (OGTT) The patient was on a general diet with frequent feedings to avoid hypoglycemia One hundred g of glucose were given orally and blood for glucose and insulin were drawn at 0 0 5 1 2 3 4 and 5 hours

Intravenous Tolbutamide Tolerance Test (IVTTT) Four tolbutamide tolerance tests were performed with an intravenous dose of 20 mg/kg (Fig 3) Blood for glucose insulin and glucagon was drawn at the appropriate intervals

TTT no 1 was performed without prior medication on 8/70 TTT no 2 was performed on 6/71 while the patient had been on diazoxide 100 mg p o twice daily for 6 months and withheld the morning of testing TTT no 3 was performed on 1/72 prior to 50% pancreatectomy Diazoxide had been maintained at the same dose for one year and again withheld the morning of testing Propranolol (2 5 mg) was given by intravenous push followed by a 30 min infusion at 0 04 mg/min Tolbutamide was injected at the beginning of the propranolol infusion Because of stupor at 30 min 25 g of glucose was given intravenously and blood for insulin glucagon and glucose was drawn after 5 and 15 min TTT no 4 was performed on 3/72 two and a half months post operatively off diazoxide As in TTT no 3 intravenous glucose was given at 30 min

Intravenous Glucagon Test To assess his islet insulin response to glucagon prior to diazoxide therapy 1 0 mg of glucagon was given by intravenous push and blood drawn for glucose and insulin at appropriate intervals

RESULTS

During the OGTT there was a brisk rise in insulin to a peak of 165 μU/ml at 60 min with a low plasma glucose value of 2 2 mmol/l at 240 min (Fig 2)

The four tolbutamide tolerance tests are shown in Fig 3, Panel A

TTT no 1—8/70 The fasting plasma glucose was 3 5 mmol/l and decreased to 1 1 mmol/l at 30 min The glucose rose to 1 9 mmol/l at 60 min and remained around that level for 180 min (not shown) The serum insulin peaked at 180 μU/ml at 5 min then decreased to a 13 μU/ml at 30 min and continued to decrease to a low of 6 μU/ml at 180 min (not shown)

TTT no 2—6/71 Diazoxide 100 mg twice daily was started 11/70 and on 6/71 the TTT no 2 resulted in hypoglycemia and stupor At baseline the plasma glucose was 3 9 mmol/l comparable to the fasting glucose during TTT no 1 The fasting serum insulin was 45 μU/ml At 3 min the insulin had increased to 520 μU/ml, dropping off to 160 μU/ml at 15 min The glucose fell to 2 2 mmol/l at 15 min and the patient became stuporous at 30 min

TTT no 3—1/72 Preoperative with propranolol infusion the baseline serum glucose was 4 6 mmol/l with an insulin of less than 5 μU/ml and the serum insulin increased to 535 μU/ml at 3 min and fell to 73 μU/ml at 30 min The plasma glucagon (not shown) initially increased to 180 pg/ml at 3 min while the plasma glucose remained constant When the plasma glucose decreased to 2 1 mmol/l the plasma glucagon further increased to 212 pg/ml When the patient became stuporous he was given 25 g of glucose intravenously (Fig 3 Panel B) The glucose rose 15 mmol/l the insulin to 470 μU/ml at 5 min The plasma glucagon (not shown) decreased to 202 pg/ml at 5 min and to 158 pg/ml at 15 min

TTT no 4—3/72 Two and a half months postoperatively the fasting glucose was 3 9 mmol/l and the insulin less than 5 μU/ml The glucose fell to 1 4 mmol/l at 30 min after tolbutamide and stupor occurred at 30 min The serum insulin peaked to 253 μU/ml at 3 min

Fig 1 (*a*) A large islet is seen budding off a duct (magnification ×84) (*b*) Multiple islets are seen budding off a duct Arrow is indicating atypical nucleus (magnification ×84)

[1] At the present time provocative tests causing hypoglycemia are performed with close monitoring of the blood glucose by at least dextrostix and optimally with a glucose analyzer

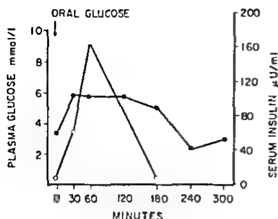


Fig 2 Glucose and insulin responses to oral glucose (100 g) prior to diazoxide ●—● glucose ○—○ insulin

and fell to 110 μU/ml at 15 min After 25 g of intravenous glucose (Fig 3 Panel B) the plasma glucose rose to 15 4 mmol/l and insulin increased to 205 μU/ml at 5 min

Glucagon Stimulation Test (Fig 4) 11/70 The basal plasma glucose was 1 1 mmol/l and insulin was 15 μU/ml Plasma glucose rose to 2 6 mmol/l after 30 min and fell to 1 1 mmol/l at 4 hours The insulin increased maximally to 80 μU/ml at 10 min The patient was asymptomatic throughout the study

DISCUSSION

The tolbutamide tolerance test of 8/70 was consistent with an insulinoma In 6/71 the patient had been on diazoxide for 6 months and his insulin response to tolbutamide was exaggerated Normally in healthy subjects diazoxide acutely lowers the basal plasma insulin and increases the blood glucose (15) In patients with an insulinoma the same phenomenon occurs to a greater magnitude (9) However although the basal level of insulin is lowered Fajans et al (9) reported that the insulin response to tolbutamide was not inhibited by prior administration of diazoxide and trichlormethiazide in three patients with islet cell tumors In fact one of these patients had an exaggerated insulin response but this was interpreted as spontaneous variation Somewhat in contrast Pagliara (14) reported a patient with islet cell hyperplasia in whom

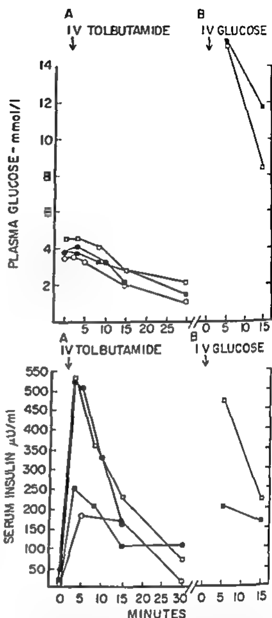


Fig 3 Panel A Blood glucose and insulin responses to intravenous tolbutamide 1 0 g ○—○ BS and IRI TTT no 1 prior to diazoxide ●—● BS and IRI TTT no 2 on diazoxide withheld the morning of testing □—□ BS and IRI TTT no 3 on diazoxide withheld the morning of testing with propanolol injection and infusion ■—■ BS and IRI TTT no 4 two and a half months post operative 50% pancreatectomy *Panel B* Glucose and insulin responses to 25 g of i v glucose given because of stupor terminating TTT no 3 and no 4 Symbols as in Panel A

diazoxide perhaps because of very high doses blunted the insulin response to tolbutamide

Thus on one hand the hyperresponsiveness to tolbutamide after treatment with diazoxide in our patient appears paradoxical On the other hand Anderson et al (1) using dogs

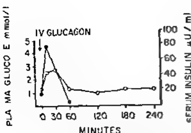


Fig 4 Glucose and insulin response to 1 mg of glucagon i v prior to diazoxide O—O glucose ●—● insulin

found that diazoxide potentiated tolbutamide stimulated insulin release and that this effect of diazoxide could be blocked by propranolol Further supporting our findings Greenwood et al (10) showed that diazoxide induced an exaggerated insulin response to tolbutamide and glucagon given simultaneously in four normal and ten diabetic human subjects Diazoxide pre treatment has also been shown to enhance the glucose induced insulin release from perfused rat pancreatic fragments treated *in vitro* (6) from perfused pancreases of rats treated chronically with diazoxide (5) and in a 15 year old patient with an insulinoma (4)

The explanation for potentiation of tolbutamide induced release in our study and of both glucose and tolbutamide induced release in other studies is not known In our patient the diazoxide effect did not appear to be beta receptor mediated (as suggested by others (1 6)) since the hyper responsiveness persisted despite propranolol administration Another possibility is that diazoxide blocks insulin release as *insulin synthesis proceeds* resulting in an increase in stored hormone After the diazoxide is withdrawn for a certain time to allow its blocking effect on insulin release to diminish a provocative stimuli such as glucose or tolbutamide as in our case causes enhanced insulin release

The increase in serum insulin with oral glucose and with intravenous glucose on termination of TTT no 3 and no 4 indicates that the hyperplastic islets were normally responsive to this physiologic stimulus Indeed the peak insulin response to oral glucose was greater than that observed in normal boys (12) and the response to intravenous glucose was almost comparable to the response to intravenous tolbutamide The greater response to intravenous glucose on diazoxide raises the possibility that diazoxide also potentiated the insulin response to glucose This response was apparently not blocked by persisting levels of propranolol after discontinuation of propranolol infusion

Two and a half months after a 50% distal pancreatectomy tolbutamide induced symptomatic hypoglycemia and hyperinsulinism consistent with the diagnosis of islet cell hyperplasia The insulin response to intravenous glucose was not as great as prior to surgery This is related to the fact that half the pancreas was removed and to the discontinuation of diazoxide which prior to surgery potentiated the glucose induced insulin release A four year follow up has not revealed any evidence for the presence of an insulinoma in his residual pancreas in addition to his islet hyperplasia

This patient also provided an opportunity to observe the glucagon responses of hyperplastic cells to tolbutamide and glucose and the insulin response to administered glucagon (Fig 4) On 1/72 plasma glucagon appeared to respond appropriately to changes in the serum glucose (See Result TTT no 3) The intravenous glucagon stimulation test (Fig 4) prior to diazoxide resulted in a normal insulin response in comparison with the normal controls of August & Hung (2)

ACKNOWLEDGEMENT

We thank Dr John Ensinck for performing the plasma glucagon assays

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CASE REPORT

TRANSIENT INTESTINAL LYMPHANGIECTASIA

HARALD ØRBECK TOVE EEG LARSEN and TORSTEIN HOVIG

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ABSTRACT Ørbeck H Larsen T E and Hovig T (Department of Paediatrics and Institute of Pathology Rikshospitalet University of Oslo Oslo Norway) Transient intestinal lymphangiectasia Acta Paediatr Scand 67 677 1978.—Intestinal lymphangiectasia may be more protean in clinical manifestations and less rare than earlier suspected A recent report points out that there are two types of the disorder one congenital and the other acquired and transitory A case is reported which fulfills the current clinical laboratory radiological and histological criteria for the diagnosis of the disease and represents the first report in Scandinavia of transient intestinal lymphangiectasia with rapid and complete recovery within a few months after initiation of MCT diet

KEY WORDS Intestinal lymphangiectasia medium chain triglycerides

Intestinal lymphangiectasia is characterised by dilatation of lymph channels in the intestinal mucosa and submucosa The condition leads to a protein losing enteropathy and is accompanied by hypoproteinaemia hypogammaglobulinaemia peripheral oedema and lymphocytopenia

Primary intestinal lymphangiectasia is assumed to be genetic in origin is a chronic disorder and treatment is likely to be life long A recent study of a large series of patients lends support to the concept that there are two types of the disorder one genetic and the other acquired and transitory (6) The evidence points to this condition being relatively common and asymptomatic with the induction of symptoms being caused by exogenous factors In the paediatric age group the condition may vary widely in its manifestation and severity In the Scandinavian literature a few reports of the disease in children have been published (2 5 9) but none refer to transient intestinal lymphangiectasia

CASE HISTORY

Girl born March 74 the first child of healthy non related parents No known hereditary disease or instances of lymphoedema in the family Normal pregnancy with an uncomplicated birth at term Birth weight 3 770 g The child was breast fed for the first three months later a supplementary milk substitute was introduced before gradual transition to a conventional solid food diet The girl thrived with normal weight increase and growth in length From two months of age the parents observed periodic puffiness under the eyes and from the age of three months she became readily irritated Swelling occurred over the back of the left hand and oedema developed on both feet The girl started to vomit refused to eat seemed more irritable and was therefore admitted to hospital at the age of 3 months

On admission the patient was pale and hyper irritable Muscular stiffness was present with pronounced carpopedal spasm and positive Chvostek s sign Oedema was found on the dorsal parts of the feet and on the soles

Physical examination revealed distinct signs of tetany Laboratory studies (Table 1) disclosed hypoalbuminaemia hypocalcaemia hypomagnesaemia and alkalosis The patient was initially treated with plasma intravenously and also given glucose plus calcium and electrolytes Two days later she received a blood transfusion and in the following 24 hrs parenteral alimentation and albumin intravenously This treatment resulted in an improvement in her condition with normalized serum

Table 1 *Laboratory results*

Laboratory test	On admission	On discharge	One year after discharge	Three years after discharge
Serum calcium (mmol/l)	1 4	2 1	2 6	2 6
Serum magnesium (mmol/l)	0 2	0 7	1 3	0 9
Serum phosphorus (mmol/l)	1 8	2 1	1 6	1 3
Total serum protein (g/l)	31–25	42	66	67
Serum albumin (g/l)	16– 8	25	36	34
Serum gammaglobulin (g/l)	3– 3		11	15
Serum IgG (g/l)	0 2		8 0	10 4
Serum IgA (g/l)	0 1		0 3	0 9
Serum IgM (g/l)	1 0		2 2	2 1
Alkaline phosphatase (U/l)	165		668	640
ALAT (U/l)	92		23	15
ASAT (U/l)	94		35	34
OCT (U/l)	250		131	6

magnesium and calcium values and an increase in serum albumin concentration to 24 g/l

At this point the child developed increasingly severe diarrhoea initially interpreted as a sign of malabsorption on the basis of coeliac disease or possibly the result of milk allergy After an unsuccessful attempt to obtain an intestinal biopsy Velactin® was given No improvement was obtained with this diet and the infant continued to have intermittent diarrhoea and oedema

More marked hypoalbuminaemia then developed and albumin infusions had to be given at intervals of a few days The patient developed watery diarrhoea and pronounced oedema and the intravenous treatment with fluids plasma and parenteral feeding had to be repeated During this period the serum albumin level declined to 14 g/l

After the patient s clinical condition had stabilized again a biopsy of the small intestine was obtained 6 weeks after admission to the hospital The histology revealed villi of normal height but lymphatic channel dilatation was observed (Figs 3 4 5) The diet was changed to a formula diet in which long chain fatty acids were replaced by medium chain triglycerides (MCT) whose fatty acids are largely transported by the portal vein and whose absorption does not appreciably increase lymph flow On this formula a rapid and striking improvement occurred with gradually increasing weight without diarrhoea and no tendency to develop oedema The serum concentrations of proteins and immunoglobulins nearly normalized and the percentage and absolute lymphocyte counts increased to normal for age

After discharge from the hospital the diet was supplemented with small but gradually increasing amounts of cereal based formula and as the infant remained free of symptoms a conventional solid food diet was ultimately adopted 5 months later At the most recent examination 3 years after leaving the hospital the patient continued to be free of symptoms increasing in weight satisfactorily with no indication of hypoproteinaemia

Laboratory findings See Table 1 and Figs 1 and 2 On admission Hb 13 1 g/dl leucocytes 9 100 with 2% bandform 55% polymorphs 43% lymphocytes The percent lymphocyte count declined later to 20 and then 4%

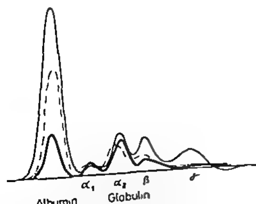


Fig 1 The pattern of serum electrophoresis of proteins on admission (– –) during hospital course (——) and three years after discharge (——)

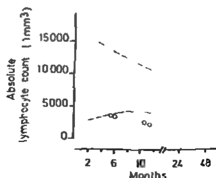
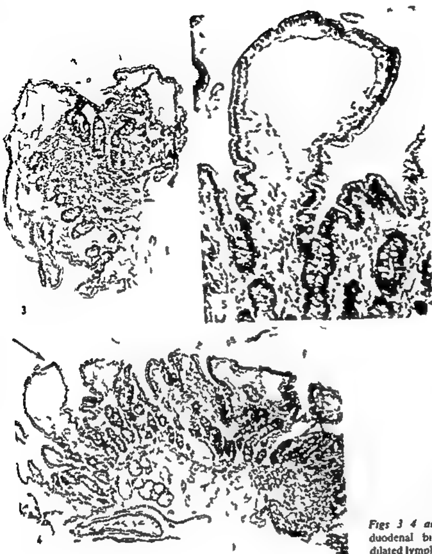


Fig 2 Lymphocyte count and age of the patient during the disease and at later controls



Figs 3 4 and 5 Light micrographs from duodenal biopsies (1974) demonstrating dilated lymphatics

Serum sodium 131 mmol/l serum potassium 3 9 mmol/l serum chloride 107 mmol/l urea 5 8 mmol/l blood glucose 7 mmol/l Ceruloplasmin 0 6 g/l haptoglobin 1 7 g/l Vitamin B 44 pmol/l Folic acid in serum and whole blood was normal Glucose tolerance test was normal Total cholesterol 4 3 mmol/l triglycerides ? 7 mmol/l Fibrinogen 3 7 g/l α_1 antitrypsin 104% of normal Pi type MZ Serum IgE was normal Faecal content of fat was normal No demonstrable eosinophilia The urine was normal without proteinuria

X ray examination revealed jejunal dilatation and segmentation of contrast media in the small intestine

Histology

Light microscopy The first two duodenal biopsies (1974) revealed intestinal villi of normal height but usually broader than normal The epithelial lining appeared normal but many of the villi contained dilated lymphatics Dilated lymphatics were also noted in the submucosal layer (Figs 3 4 5)

In the biopsy material from 1975 and 1977 the villi appeared normal but in the submucosal layer dilated lymphatics were occasionally seen In all biopsies lymphocytes plasma cells and some eosinophil granulocytes were present but their number did not appear to be increased

Electron microscopy A liver biopsy was obtained for electron microscopy in 1975 The hepatocytes were well preserved In some of the cells however lipid vacuoles and vacuoles containing an amorphous material were noted (Fig 6) The appearance of these vacuoles was very similar to that observed in α antitrypsin deficiency

Duodenal biopsy material obtained in 1977 has been examined both in scanning and transmission electron microscopy No characteristic ultrastructural changes were observed

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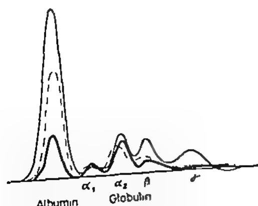


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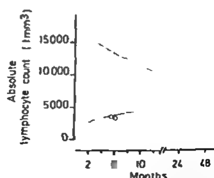


Fig 2 Lymphocyte count and age of the patient during the disease and at later controls

DISCUSSION

Protein losing enteropathy is a characteristic syndrome with varying etiology Primary lymphangiectasia formerly known as idiopathic hypoproteinaemia is a rare cause However this condition should be suspected when a patient belongs to the younger age group and has peripheral asymmetric oedema and a markedly low level of serum proteins that cannot be explained on the basis of proteinuria or liver disorder Demonstrable lymphocytopenia is an important sign of intestinal lymphatic anomaly Our case conforms to the classical picture of lymphangiectasia during a critical period The patient s reduced lymphocyte count and reduced levels of serum albumin and gammaglobulin indicated losses of total lymph Because of the child s age no attempt was made to demonstrate pathological leakage of plasma proteins to the intestine by means of isotopes Instead verification of the diagnosis was made by biopsy of the small intestine

Tetany was initially a dominant sign in our patient but was noted in only 12% of the patients in a recent survey (1) The underlying hypocalcaemia may be due to a loss of calcium to the intestine or to reduced absorption of calcium and vitamin D Chylous effusions are often seen and individual instances of temporary blindness have been observed resulting from maculaoedema The gastro-intestinal symptoms are normally moderate and consist of intermittent diarrhoea with lowgrade steatorrhoea in one out of five cases however the diarrhoea is more serious

The primary hereditary type of intestinal lymphangiectasia is a distinct disease with congenital hypoplasia of the lymphatic system often associated with anomalies outside the gastro-intestinal tract Obstruction of lymph drainage from the intestine can lead to secondary intestinal lymphangiectasia either functional as in constrictive pericarditis or a direct result of organic lymph blockage as in lymphoma leukemia Whipple s disease and retroperitoneal fibrosis

In addition to the normal passive leakage a total plasma loss to the intestinal tract occurs with loss of proteins of all molecular sizes together with lymphocytes

In particular the relatively small albumin molecules are excreted but also globulin and especially gammaglobulin suffer the same fate Hypoalbuminaemia occurs when the liver s maximum protein synthesising capacity is exceeded Up to twice the normal synthetic capacity may be induced to compensate for increased protein loss Generally with normal liver function the loss must be greater than 0 4 g/kg body weight per day before hypoalbuminaemia develops

Electron microscopic investigation of a liver biopsy from our patient showed prominent protein deposits in cisternae of the endoplasmic reticulum which can denote increased protein synthesis Typical changes in the concentrations of serum proteins indicate that the protein synthesis is insufficient to compensate for the protein loss Albumin and gammaglobulin are usually markedly reduced and transferrin and ceruloplasmin moderately reduced but fibrinogen is usually present in normal concentration (7)

Since T lymphocytes recirculate from the bloodstream to the tissues and thence via the lymphatic system back to the bloodstream they are more vulnerable to gastro intestinal chylous leakage than are the B cells which have a shorter survival and which tend to remain in the peripheral circulation Investigations indicate that lymphocyte loss occurs selectively at the expense of the T lymphocytes (8)

Even though the main class of immunoglobulin molecules shows a decline in concentration in the serum the humoral immune response still functions on exposure to antigen Diminished cellular immunity is a consequence of lymphocytopenia demonstrated by weak skin reactions on exposure to antigens and reduced ability to reject homografts (4)

Our investigations did not reveal a second

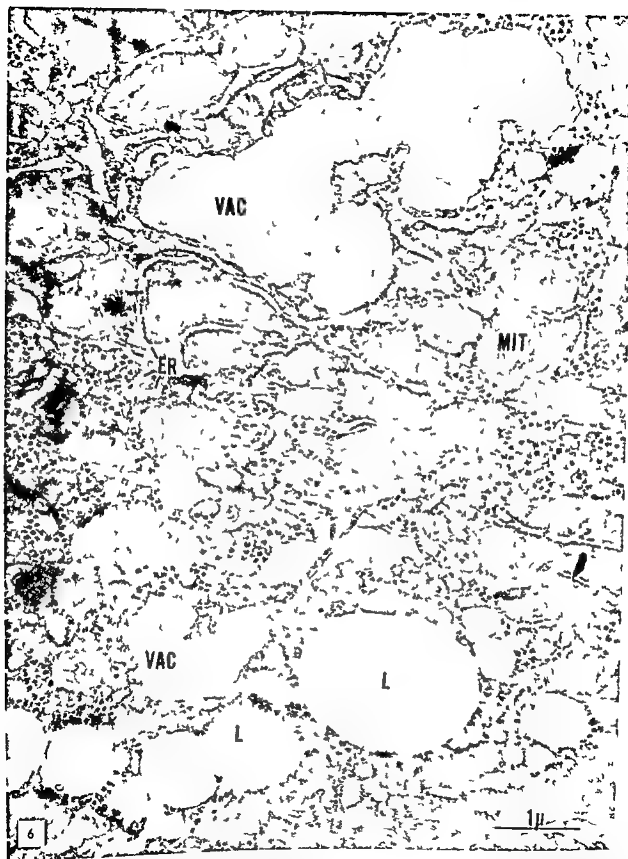


Fig 6 Electron micrograph demonstrating hepatocyte with lipid material (*L*) and vacuoles containing amorphous material probably representing dilated cisternae of endoplasmic reticulum *ER* endoplasmic reticulum *GLY* glycogen *L* lipid MIT mitochondria VAC vacuole

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REVIEW ARTICLE

CHILD ABUSE A LITERATURE REVIEW

D LAGERBERG

From the Department of Paediatrics University Hospital Uppsala Sweden

ABSTRACT Lagerberg D (Department of Paediatrics University Hospital Uppsala Sweden) Child abuse A literature review Acta Paediatr Scand 67 683 1978 —The following is a summary of a recently published review of Swedish and international literature concerning children exposed to abuse and neglect or manifesting failure to thrive The review is the first report appearing from the so-called PRU group in Uppsala—Psycho-social Risk Children in Uppsala The group is a multidisciplinary team engaged in a research project about Uppsala children exposed to mental social and physical risks in their homes The project is financed by the Save the Children Fund which has also contributed to the printing costs of the book Various professions are represented in the research team namely social work family therapy child psychiatry child psychology paediatrics and sociology The complete review was published in the autumn of 1977 under the following title Dagmar Lagerberg/PRU-gruppen Foraldravåld mot barn En litteraturstudie (PRU group Child abuse by parents A literature review) A second main report presenting the aims material methods and results of the project is planned for publication in 1979

KEY WORDS Battered baby syndrome battered child syndrome child abuse and neglect failure to thrive maltreatment syndrome of childhood non accidental injury to children physical abuse of children traumatic injury in children violence against children

The principal aims of the literature review was to survey the different etiological theories and to discern how different researchers have tackled the problems of therapy and prevention in the field of child abuse Another aim was to analyse the cases for and against coercion in social work with parents and children In addition to this some other related phenomena were discussed

CONCEPTS OF CHILD ABUSE

Child abuse is not simply a matter of physical injury Injuries may also be mental or psychological Further violence against children may take an *active* or a *passive* form Active violence occurs when parents or guardians by their explicit actions abuse or neglect a child physically or mentally Violence may be said to be passive on the other hand when the child is in some way insufficiently protected from harm—without there being any intention on the part of the parents or guardians to cause harm to the child

The different forms of child abuse may be illustrated by the simple pattern in Fig 1

Fig 1 is important because it indicates the complexity of the problem of children at risk Most of the literature reviewed deals with active physical injury and for this reason these aspects are emphasized in the present summary The research of the PRU team however is mainly concerned with the opposite—passive mental injury with a risk of developing unfavourably due to damaging factors in the home such as parents marital crises unemployment drug or alcohol addiction etc

HISTORY

The classical international literature dealing with what has later been called the battered

ary cause for dilated lymphatics One reference has been made in the literature to histological findings corresponding to intestinal lymphangiectasia in some instances during intravenous alimentation for protracted diarrhoea in infancy (3) The sequence of clinical events makes this explanation in the present case improbable The clinical course the laboratory findings and the morphological appearance in the case reported indicate the existence of a transitory type of intestinal lymphangiectasia

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	Physical	Mental
Active		
Passive		

Fig 1 Different categories of injury

child syndrome appeared in the 1940s Caffey (5) was the person who first recognized the co appearance in small infants of soft tissue swellings and cortical thickenings in the skeleton From later investigations by the same author (6 7 8) the traumatic nature of this condition became evident while primarily it was ascribed to obscure internal causes Other reports concerning this problem include those by Kempe et al (20) Gil (13 14) Kempe (18) Kempe & Helfer (19) Helfer & Kempe (15 16) and Lynch (23)

In Sweden the child abuse problem was first recognized about one decade later by Selander (31 32 33) Further contributions were made by Frisk (12) Official counselling and regulations concerning child abuse have been issued by the Swedish National Board of Health and Welfare (27 29) The Board has also undertaken research of its own (28 30)—in the latter case jointly with the All manna Barnhuset a research institute for child care problems

INCIDENCE

The real incidence of child abuse is not known This phenomenon naturally enough occurs in the absence of witnesses However many authors have tried to calculate or estimate figures but these figures differ widely indicating how very little we know about the magnitude of the problem One Swedish report based on hospital cases gives a yearly incidence of physical abuse—cases occurring per million population—of 1 6 children under 18 (28) On the other hand statistical estimates combined with interview data from the USA have yielded an annual incidence of over 2000 children under 18 per million population (22) The difference is enormous even if we bear in mind that the USA calculations are not restricted to hospital cases The fact is that the true occurrence of abuse and neglect is almost entirely unknown What we really see is the top of the iceberg In addition to the registration problems the matter is further complicated by difficulties of definition

ETIOLOGICAL MODELS

On review of the literature we find that the factors proposed as explanations for physical abuse neglect and failure to thrive in children can be grouped into three main categories or etiological models

Individual and family

Researchers with this view hold that violence against children and related phenomena result from individual characteristics of the parents or family characteristics for instance marital difficulties Other characteristics assignable to this model are emotional disturbances in the parents mental and physical illnesses intellectual deficiencies socio economic problems such as unsatisfactory housing or unemployment antisocial behavior such as alcohol or drug addiction criminality violence between adults and isolation Among several others Kempe (18) focused attention on this group (emotional disturbances) Steele & Pollock (35) have also emphasized the damaging influence of criticism excessive demand and lack of caring love in the early lives of the abusing parents Smith (34) has thoroughly

explored psychiatric psychological and social characteristics of abusing parents

Events

Another special category is what we call the events model where it is assumed that something happens some event occurs that causes a breakdown in the relationship between mother and child In unfortunate cases the end result of such a breakdown may be neglect or abuse Lynch and collaborators have devoted special interest to this explanatory model The following six factors—or "events as we may call them—have been discerned each of which show a statistically significant relationship with child abuse (23)

abnormal pregnancy
abnormal labour or delivery
neonatal separation
other separation in the first six months
illnesses in the first year of life
illness in the mother in (the child s) first year of life

The author uses non injured siblings as controls thereby correcting for social influences The above six factors appear to correlate with increased violence against the affected child especially when they occur in conjunction and in a previously vulnerable family Lynch s interpretation is that the factors cause a bonding failure between mother and child which ultimately results in abuse

Structural factors

A third etiological model is suggested by authors who stress the influence of environmental factors—cultural social and economic This view is represented by Gil (13 14) who considers physical abuse to be mainly a result of structural factors for instance culturally sanctioned interpersonal violence in child rearing different child rearing norms in varying social ethnic and national groups and chance environmental factors The part played by individual characteristics and shortcomings according to Gil is secondary Individual characteristics consequently would not cause violence against children if not reinforced by structural factors The remedy proposed by Gil is to eliminate poverty and unfavorable living conditions in the population at large

In our opinion no single model can be considered the true one the one which explains everything Certainly the most fruitful and constructive way is to apply a combination of all three models laying emphasis on one or the other according to the characteristics and circumstances of the particular case

ADDITIONAL CONCEPTS

Some additional concepts may be useful in classifying cases of child abuse

Early causes–late causes Emotional stresses in the parents own childhoods are early causes as opposed to stress and disasters occurring in later life perhaps immediately before the incident of abuse

Predisposing causes–precipitating causes A chronic disease or chronic unemployment may be a factor predisposing for the occurrence of child abuse whereas alcohol intoxication a family row or a sudden additional stress are examples of precipitating factors (immediate causes of abuse)

General causes–specific causes This distinction has to do with the fact that some but not all cases of child abuse or neglect are due to largely intelligible factors— general factors—which can be easily observed in the family s life situation and are generally known to foster risk situations In these cases all children in the family tend to be equally affected The problems do not primarily concern the relationships or bonds between parents and children but have to do with other troubles such as economic difficulties or alcohol addiction

Specific causes on the other hand play a part only in individual cases and in different ways in different cases—for instance when a mother is unable to cope with her relation

ship to one special child mainly because the child means something to her, for example by being very much like herself or the other parent Siblings may be unharmed The relationship between parent and child is affected in a specific way that may bring about neurotic and even psychotic behavior

RISK CRITERIA

Some authors have tried to define recognizable criteria by which parents and children at risk may easily be identified Two main classes of criteria can be distinguished—characteristics of the parents and characteristics of the child Since many studies focus interest primarily on the parents it ought to be mentioned here that an excellent account of child abuse from the victim s perspective—i e that of the child—has been given by Martin (25)

PARENT CHARACTERISTICS

Parents may for instance be over anxious about the health of a quite normal child or the mother may raise concern already in the maternity ward about her lack of capacity to care for the child Sometimes psychological tests are used to disclose aggressive tendencies, impulsivity and lack of self control In some instances parents show inadequate concern about a child s failure to thrive Interviews with the parents may reveal emotional stresses in their own early lives Generally speaking the following sample of criteria can be regarded as typical warning signs

negative emotional reactions connected with the baby
inability to form an emotional bond with the child
diffuse i e interlocked social problems that are difficult to define where the remedy is not apparent
complications during pregnancy delivery or the child s first year of life
vulnerable periods especially connected with changes in family life circumstances
negative patterns of interaction within th family

When an injury has already occurred th following well recognized criteria may be ind: cations of abuse

The parents wait for several hours befor seeking medical advice
the parents explanation of what has happene to the child seems improbable

CHILD CHARACTERISTICS

Typical features by which children at risk can be identified are

poor hygiene
undernourishment
subnormal height and/or weight
weight gain during hospital stay (in failure to thrive children)
mental retardation
language retardation
depression
behavioral disorders
signs of excessive mistrust
the child smiles at anyone or accepts help from anyone
extreme and premature adaptation to parental needs

Some authors e g Ayoub & Pfeifer (1) and Coombes et al (9) have tried to construct indices by combining several different factors

PROGNOSIS

Studies undertaken to determine the prognosis for children who fail to thrive have revealed a considerable risk of long term sequelae for instance emotional disturbances and mental and language retardation (4 11) In some cases however no disturbances were observed in children with previous failure to thrive Koel (21) has devoted attention to the continuum of failure to thrive neglect and

ultimately physical assault and his observations are also of vital prognostic importance

There is some disagreement about the future prognosis of physically abused children This has to do with the authors different etiological outlooks and with their inclination toward optimism or pessimism Furthermore the criteria for a good prognosis vary according to whether the perspective is narrow or wide Thus with a narrow perspective the interest is restricted to whether or not the child will be attacked again whereas with a wide perspective account is also taken of the overall emotional intellectual and social development of the child

Studies show that physical abuse tends to be repeated and also leads to increasingly severe injuries (2 3 12 20 28 32) It has also been demonstrated that it may produce long term mental and physical sequelae (2 10 33 36)

PREVENTION AND TREATMENT

Opinions and recommendations concerning the prevention and treatment of violence against children vary considerably To a certain extent this is due to differences in the presupposed etiological models Explanations based on individual characteristics naturally favor recommendations involving supervision therapeutic programs separation of parents and children and placement in a foster home Structurally oriented explanations on the other hand elicit social implications such as economic reforms changes on the labor market and so on It is then assumed that such changes will benefit everyone and therefore also prevent child abuse and neglect

In recent years there has been a slight trend away from emphasis on individual measures in prevention and treatment (mainly foster family placements) toward structurally oriented recommendations The National Board of Health and Welfare (30) for instance in addition to individual measures—which will certainly remain necessary—deals extensively with general prevention in structural terms

In any case the problems arising from this difficult issue are far from settled Some authors consider individual action—separation of parents and child—as the only safe remedy while others are more inclined to let the child stay with his biological parents under the supervision of social authorities In fact two distinct views can be discerned—the pessimistic view and the optimistic view

The pessimistic view holds that violence against a child once having occurred implies too many risks to let the parents have another chance to care for the child on their own Accordingly some radical measure is necessary i e placement of the child in a foster home The child cannot be safely brought back to his biological parents The main task of the doctor and the social worker should be to protect the child

According to the optimistic view there is a good chance that child abuse and neglect may be prevented even if the child is allowed to stay with his parents provided the family is given adequate help and support Therapeutic measures should consist in restoring some of the emotional deprivation which these parents are assumed to have suffered when they themselves were children Consequently a form of mothering care should be provided accepting the parent without criticism and allowing her or him to become emotionally dependent for a period of time The ultimate aim is to make the parent capable of growing more resourceful and mature Meanwhile the child may temporarily be placed away from home

Kempe (18) and Lynch et al (24) report relatively successful results with abusing parents under their care (in the latter study however the material also included persons other than abusing parents) In 80% and two thirds of the cases respectively enough improvement occurred in family functioning to allow the child to be at home It should be noted however that the therapy provided in

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these cases was intensive and time consuming It was given on an in patient basis and lasted for several weeks 24 hours a day through every day of the week

THE CASE FOR PESSIMISM

What are the reasons for maintaining a pessimistic view? Primarily there is the risk of recurrent—increasingly serious—violence and of mental and physical sequelae These risks are scientifically documented On the other hand they might perhaps have been reduced if the parents had been offered adequate therapy at least in some cases

But there is also a risk that therapy will focus entirely on parental needs leaving aside the child s emotional requirements and the general family climate These important factors may be overlooked if the therapist s aim is restricted to preventing incidents of actual violence without emotional improvements Recent research confirms this possibility (2)

Finally physical injury is not a negligible matter Many authors feel justified in adopting the view that however small the risk it must be a primary duty to minimize it and therefore stick to a pessimistic view

THE CASE FOR OPTIMISM

As mentioned earlier Kempe and Lynch found that the majority of parents under their care proved to be treatable at least in the sense that renewed violence was prevented This fact speaks for optimism Optimism furthermore appears as a more sympathetic attitude than pessimism It seems to foster sensible decisions made in consideration of the family s individual situation and special characteristics

There is also a risk that coercive measures and supervision by social agencies may destroy all that the parents have left of self reliance and a capacity to cope with difficulties Furthermore it is practically impossible to tell what is after all in the best interest of the child and what exactly will be the outcome of a specific measure (26) Therefore since in uncertain cases the best thing to do must be to cause as little damage as possible it may be wiser to leave the child at home supporting the family in other ways This of course does not apply to those clear cut cases where the child is really known to run a substantial risk of injury or ill health

There is also a strong argument to the effect that foster placement may cause damage to a child (26) Studies show that many children suffer in their foster homes overwhelmed by separation anxiety and feelings of guilt and so on One researcher Hook (17) holds that these children cannot benefit from foster care without previous individual and intensive psychotherapy Secondly the child can never acquire a natural status in the foster home He or she becomes exposed to serious identity and loyalty conflicts At the same time foster parents are not supposed to grow too attached to the child which may in turn unfavorably affect the child s feeling of security and emotional development Finally foster family placements are by definition unstable The child has no possibility of knowing how long he is to stay with the foster parents

CONCLUDING REMARKS

The review of the literature on violence against children summarized in this article was initiated to provide a theoretical basis for our future work We have seen that physical violence is the most common subject of research concerning children at risk Its incidence is not known Its causes are multifacetted and complicated The controversy between the pessimistic and the optimistic therapeutic views is applicable not only to children who have been physically attacked but also to children exposed to more comprehensive suffering We know that a large number of children suffer in their homes thus falling into the category of children at risk The problems are challenging especially since

there seems to be good arguments both for and against each of the two views—pessimism and optimism It is hoped that the second PRU team report based on an empirical Uppsala material will illuminate some of the pertinent questions

ACKNOWLEDGEMENT

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BOOK REVIEWS

I R E Gordon & F G M Ross *Diagnostic radiology in paediatrics* 384 pp illus *In* Postgraduate paediatrics series (ed John Apley) Butterworths London Boston 1977 £29 50 ISBN 0-407-00171 ?

The volume belongs to a Postgraduate paediatrics series and is intended not only for paediatricians but also for trainees in paediatric radiology Of its nine sections the first three are concerned with the skeletal system which in addition takes a major part of a final section devoted to growth and development Fractures and other traumatic lesions have however received only little space Other sections deal with the cardiovascular the respiratory the gastrointestinal and the urinary organs respectively and one with the central nervous system

Except for traumatology the text and the numerous illustrations display most of the radiologically recognizable abnormalities commonly seen in infancy and childhood A wide selection of rare or uncommon conditions is also included Some of these are described in detail but others only superficially and some are cursorily mentioned as differential diagnoses without being otherwise dealt with Such disparities may have been inevitable because of the limitation of space which has also necessitated omission of many unusual but important abnormalities In some sections a partly different choice of malformations for example might have been preferable but this is to some extent a matter of personal taste

The main radiologic special procedures are briefly described and discussed Most of these may be applied in several ways and different modifications may be equally useful Those recommended by the authors call for no special comments except that few paediatric radiologists are likely to accept subcutaneous injection of urographic contrast medium Various other technicalities such as some of the radiologic measurements referred to are mentioned without explanation of the procedure and certain descriptions in this context are not quite accurate e g that of the Cronqvist skull index

Despite such minor inadvertences the book is recommended as an introduction to the application of radiology to paediatric problems It will be valuable reading also for the specialist in general radiology and even for the paediatric radiologist as an adjunct to more comprehensive or specialized textbooks The paediatrician on the other hand might consider the volume unnecessarily laborious since in Scandinavian hospitals for example much information on the radiologic procedures and findings is easily obtained at the frequent clinical sessions held in the radiologic department In countries without such a radiologic routine service a text of this kind should however be a great help to the paediatrician by facilitating the important close integration of the clinical and radiologic aspects of his specialty

Georg Theander

Isabelle Valadian and Douglas Porter *Physical growth and development* 540 pp illus Little Brown and Company Boston 1977 ISBN 0-316-89525 3

There has been a great need for a clearly written and stimulating book on physical growth and development from conception to maturity This self instruction book is easy to read but rather voluminous It is written for students of different backgrounds From the paediatric student s point of view about one fourth of the material is too elementary

Each chapter represents a short review of the anatomy and physiology of the organ followed by an often excellent presentation of the development and function of the system A test that follows each unit controls the learning outcomes The text does not cover social behavioural and psychological development

This excellent book can be highly recommended especially to teachers of pediatrics and students preparing for a variety of health careers

Tomas S eger

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ANNOUNCEMENT

NATHALIE MASSE RESEARCH FELLOWSHIP AND PRIZE

Dr Nathalie Masse who died in 1975 was director of teaching at the International Children s Centre for 18 years She made major contributions to the improvement of child health internationally

In 1976 many friends of Dr Nathalie Masse established a Memorial to perpetuate her memory to fulfil this purpose a fellowship and an international prize were created

1 *The Nathalie Masse Research Fellowship*
This fellowship (granted in even numbered years) has just been awarded for the first time for a research on nutrition education in a socially deprived region of South America

The next fellowship to be awarded in 1980 is likewise intended for young research workers It is intended to help them in their projects directed to problems in social and preventive pediatrics

2 *The International Nathalie Masse Pri e*
This prize amounting to 10 000 French fran s wi awarded for the first time in 1979 and every 2 years ther after

It is intended as compensation for an original work co cerning childhood prepared by an institution or an in vidual under 40 years of age with a view to encourag studies by young professionals or research workers

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Acta Pædiatr Scand 67 693–697 1978

TRANSCUTANEOUS P_O MONITORING IN NEONATAL INTENSIVE CARE

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ABSTRACT Lofgren O Henriksson P Jacobson L and Johansson Ö (Department of Obstetrics and Gynecology and the Department of Paediatrics Allmanna Sjukhuset University of Lund Malmo Sweden) Transcutaneous P_O monitoring in neonatal intensive care Acta Paediatr Scand 67 693 1978 —The transcutaneous P_O monitoring technique was applied [redacted] 20 newborns The method proved reliable during hypoxemia normoxemia and hyperoxemia with a high correlation between P_{tcO_2} and P_{aO_2} in simultaneously obtained arterial samples Although P_{tcO_2} reliably reflects changes in P_{aO_2} occasional arterial samples are still required for establishing the relationship between P_{tcO_2} and P_{aO_2} especially in patients with impaired circulation When this relationship has been determined the therapist may rely on the recorded P_{tcO_2} level for hours given that the energy supply required [redacted] maintain the electrode at a preset temperature level remains constant A considerable difference between P_{tcO_2} and P_{aO_2} or a change in the energy supply level to the electrode may alert the therapist to check the patient s circulatory status The P_{tcO_2} technique is now fully developed and can be recommended for use in neonatal intensive care

KEY WORDS Transcutaneous P_{O_2} monitoring intensive care newborns

Studies of the P_{tcO_2} monitoring technique in neonatal intensive care either with the original equipment built by Huch et al (9) or with modified equipment (3 4 7 8 15 16) have usually reported highly statistically significant correlations between P_{tcO_2} and P_{aO_2} (3 4 6 7 8 10 11 13 16 20) However the reliability of the method during hypoxemia and hyperoxemia (11 17 20) and in conditions with impaired peripheral circulation (6 18) has not yet been proved The present study was performed in order to evaluate the reliability of th[redacted] P_{tcO_2} monitoring technique during various conditions in a series of newborns

EQUIPMENT AND PROCEDURE

The equipment used was a Radiometer TCM 1 unit (Radiometer Denmark) A 17 μ polypropylene membrane was used The electrode temperature was set at 44 5 C (anode temperature the skin temperature was assumed to be 43 5 C) Immediately before and after every recording the electrodes were calibrated and recalibrated in an antiseptic water solution of Urolicide® (American Latex Cooperation Indiana USA) bubbled through with air The temperature of the calibration solution was 43 5 C Two independent sets of the equipment were used to obtain simultaneous recordings in most patients The electrodes were applied in the subclavicular area on either side of the sternum P_{tcO_2} was recorded for periods of up to 10 hours with a mean recording time of 3 hours (Table 1) The oxygen concentration in the incubator was altered stepwise several times during every recording The oxygen concentration of the respired air was measured with an oxygen analyzer (Ivac 1725 AGA Sweden) Arterial blood samples were drawn from indwelling umbilical artery catheters at each oxygen level after the P_{tcO_2} level had stabilized The tip of the catheter was placed at the level of L II–L III (X ray) P_{aO_2} was analyzed with Radiometer AME 1 equipment (Radiometer Denmark) immediately after sampling

PATIENTS

Recordings were performed in 20 consecutive patients referred to the neonatal intensive care unit because of immaturity respiratory problems or perinatal asphyxia (Table 1) Five of the seven patients with IRDS were treated with continuous positive airway pressure (CPAP) In three of these five patients a pneumothorax was diagnosed Separate case reports are provided for two patients with extremely reduced peripheral circulation All patients were normothermic during the recording period

Table 1 *Patients*

No	Gest age (weeks)	Birth weight (g)	Diagnosis	Reg time (min)	Drift kPa Left elec trode	Right elec trode	Art samples (no)
1	36	2 930	IRDS	122	0 40		5
2	36	2 400	Postnatal asphyxia	182	2 66	0 80	7
3	41	4 200	Perinatal asphyxia	73	1 33	1 33	7
4	35	[illegible] 000	Postnatal asphyxia	170	0 80	0 53	5
5	39	3 500	Heart malformation Postnatal asphyxia	197	0 67	1 06	7
6	35	2 500	IRDS (CPAP)	180	1 46	0 53	6
7	34	2 100	IRDS	248	0 13	1 60	5
8	30	1 200	IRDS	180	0	0 93	2
9	33	1 320	Immaturity Gemini I	182		0 27	6
10	41	2 700	Postnatal asphyxia	191	0 13		5
11	36	2 260	IRDS (CPAP) Pneumothorax	620	[illegible]		7
12	39	3 390	IRDS (CPAP) Pneumothorax	229	1 06	4 52	9
13	35	2 850	IRDS (CPAP)	300	0 93	0 53	7
14	30	1 500	IRDS	200	0 93	1 06	[illegible]
15	38	3 040	Gemini II Twin to twin transf with respiratory distress	170	0 27	2 13	4
16	33	2 000	IRDS (CPAP) Pneumothorax	180	0 67	0 53	4
17	41	2 870	Perinatal asphyxia	77	0 40	0 93	5
18	32	1 550	Immaturity	80	4 79	0 40	5
19	38	3 900	Septicemia? Respiratory distress	84	0 40		5
20	29	1 190	Immaturity	90	0 40	1 06	3
Mean	35 6	2 472		186	1 02	1 14	5 6
Range	29–41	1 190–4 200					

RESULTS

The time for the calibration procedure was about 2 min If zero calibration was performed the total calibration time was less than 5 min As shown in Table 1 the mean electrode drift was low although some extreme drifts were obtained

A total of 112 arterial samples was obtained (mean 5 6 samples/patient) As recordings were performed with simultaneously operating electrodes in 15 patients these 112 arterial samples resulted in 196 correlation points between P_{tcO_2} and P_{aO} (Fig 1) P_{aO} ranged between 4 5 kPa and 32 45 kPa (34 and 244 mmHg) with a mean of 12 97 kPa (97 5 mmHg)

In order to investigate the relationship between P_{tcO_2} and P_{aO} at hyper and hypoxemia three groups were formed hypoxemia ($P_{aO_2}<$ 9 31 kPa (70 mmHg)) normoxemia (9 31 kPa (70 mmHg)$\leq P_{aO} \leq$13 30 kPa (100 mmHg)) and hyperoxemia ($P_{aO_2}>$13 30 kPa (100 mmHg))

The mean difference between P_{tcO_2} and P_{aO_2} was calculated for each group The difference between P_{aO} and P_{tcO} was small in the hypoxemia and normoxemia groups with a very low mean difference between these two parameters during hypoxemia In the hyperoxemia group however this difference was numerically greater (Table 2)

In all patients the electrode temperature produced a red mark in the measurement area This red mark disappeared within the next 12

Table 2 *Difference between P_{tcO} and P_{aO_2} in various levels of oxygenation*

n=correlation points

	[illegible]$_{O_2}$ <9 31 kPa	13 3 kPa →P_{O_2} →9 31 kPa	P_{aO_2} >13 3 kPa	Total
Mean	+0 03	−0 93	−3 07	−1 45
S D	1 01	1 32	2 75	2 [illegible]
n	39	99	58	196

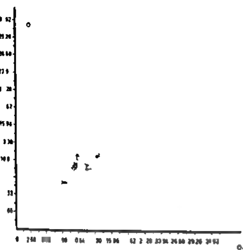


Fig 1 Correlation between P_{aO_2} and P_{tcO_2}. Larger dots indicate more than one value

hours in all patients except one case 14 a boy born in the 30th gestational week who developed a blister at the measurement site after ?00 min of monitoring The blister was completely healed within a couple of days

CASE REPORTS

Case 11 Boy born in the 36th week of gestation The gestation was presumed to be a little shorter and the boy was delivered by caesarean section due to preterm labour and breech presentation Birth weight was ?60 g The patient developed IRDS and CPAP treatment was initiated at 3 hours of age The course was uneventful until 45 hours of age when the boy suddenly developed severe respiratory difficulties with cyanosis and signs of decreased peripheral circulation due to an unilateral pneumothorax P_{tcO_2} monitoring was started prior to aspiration of the pnemothorax An arterial sample showed pH 6 69 P_{aO_2}=5 54 kPa (41 mmHg) and P_{tcO_2} =13 57 kPa (10? mmHg) while the patient was breathing pure oxygen Simultaneously P_{tcO_2} was 5 3? kPa (40 mmHg) The P_{tcO_2} level began to increase ?0 sec after evacuation of the pneumothorax as seen in Fig 2 but dropped shortly thereafter Repeated evacuations resulted in a prompt but temporary rise in the P_{tcO_2} level (Fig 2) The P_{tcO_2} monitoring clearly indicated that the patient had a communicating pneumothorax with a substantial leakage of air and a continuous pleural drainage was applied This resulted in a steady incline of the P_{tcO_2} level which was also reflected by the infant s improved general condition with normalization of the skin colour and circulation During the following hours the P_{tcO_2} monitoring technique proved helpful as an early indicator of the need for modification of the position of the intrathoracic catheter Fig 3 depicts the correaltion between P_{aO_2} and P_{tcO_2} obtained during the first P_{tcO_2} measurement period which lasted 5 hours after the diagnosis of the pneumothorax

Case 19 Full term boy normal vaginal delivery birth weight 3900 g The labour was uneventful but shortly after birth the infant showed signs of decreased skin circulation with a grey dusky skin colour During the following hours the infant showed signs of respiratory distress with slight tachypnea and intercostal retractions Pulmonary X ray was normal Septicemia was suspected but could not be verified The boy had a normal P_{aO_2} while breathing air P_{tcO_2} monitoring was started when the child was ?4 hours old and still showing the same dusky skin colour Five arterial samples were taken at different oxygen concentrations in the respired air The obtained P_{tcO_2} values were consistently about 7 98 kPa (60 mmHg) lower than the corresponding P_{aO_2} values (Fig 3) The symptoms disappeared during the second day of life and no explanation for the impaired skin circulation was found

DISCUSSION

The mean electrode drift was low but some extreme drifts were obtained However another study by Lofgren (16) raises doubt as to whether the recalibration value reliably reflects the true electrode drift during measurement

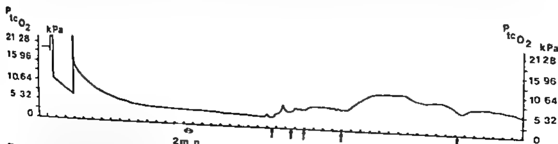


Fig ? P_{tcO_2} recorded in a child with a communicating pneumothorax Arrows indicate intermittent evacuation of the pneumothorax

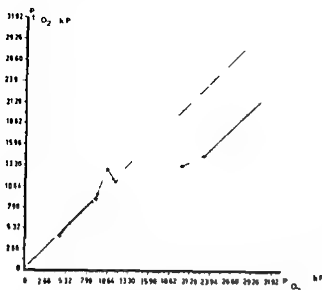


Fig 3 Triangles depicts the correlation between P_{tcO_2} and P_{aO_2} obtained in patient (11) Dots depicts the correlation between P_{tcO_2} and P_{aO_2} in patient (19)

The P_{tcO} monitoring technique combines two major advantages in comparison with other available techniques It is a non invasive method and it provides continuous monitoring Thus the known hazards associated with umbilical catheters i e thromboembolic complications sepsis (1 14 19 22) and blood loss due to frequent sampling are avoided Intermittent blood sampling from arterial catheters or particularly from puncture of peripheral arteries may cause changes in the respiratory pattern which result in P_{aO} values which are not representative for the steady state (16) The results of the current study indicate that P_{tcO_2} closely follows alterations in P_{aO} These findings agree with those reported by others (3 4 6 7 8 10 11 12 16 20) The difference between P_{tcO_2} and P_{aO_2} was however numerically greater during hyperoxemia than during normoxemia or hypoxemia but this must be considered to be of minor practical importance Measurements in two patients with impaired skin circulation also yielded a high correlation between P_{tcO_2} and P_{aO} For one of the patients (case 11) the recorded P_{tcO_2} was approximately equal to P_{aO_2} at every measurement point while in the other patient (case 19) the recorded P_{tcO_2} level was consistently about 7 98 kPa (60 mmHg) lower than the P_{aO_2} level (Fig 3) These observations illustrate the dependence of the correlation between P_{tcO_2} and P_{aO_2} on the net effect of vasoconstrictive stimuli and the vasodilatation produced by the heating element built into the electrode If the vasoconstrictive stimuli supersede the recorded P_{tcO_2} values may be discordant with the P_{aO_2} values although *changes* in the central P_{aO_2} level are clearly shown Thus the relationship between P_{tcO_2} and P_{aO_2} may be constant even in patients with a decreased skin circulation Similar results have been reported by others (17) although some findings (6 18) are discordant with those reported here Until further experience has been gained the P_{tcO} monitoring method will still require arterial blood sampling but the number of blood samples required for checking P_{aO_2} is minimized A considerable discrepancy between P_{tcO} and P_{aO} indicates an impaired skin circulation and should alert the therapist s attention to the central circulation of the patient Changes in the energy supply level should also alert the therapist to check not only the P_{aO} level by an additional arterial sample but also the circulatory status of the patient

As a normal effect of the electrode temperature a red mark is obtained at the electrode application site This should not be considered an iatrogenic injury but a natural effect of the arterialization temperature In fact the absence of such a red mark might indicate that the arterialization temperature was not sufficient The electrode temperature always implies the risk of producing burns (5) The appearance of one second degree burn in the current material concurs with the frequencies reported by others (13) Lowering the electrode temperature by 0 5–1 0°C will result in only a slight decrease in the sensitivity of the electrode (15) A slightly lower electrode temperature might therefore be used particularly in immature infants In mature infants with normal skin circulation the electrode temperature of 44 5 C is recommended

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PLASMA CONCENTRATIONS OF DIAZEPAM AND N DESMETHYLDIAZEPAM IN NEWBORN INFANTS AFTER INTRAVENOUS INTRAMUSCULAR RECTAL AND ORAL ADMINISTRATION

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ABSTRACT Langslet A Meberg A Bredesen J E and Lunde P K M (Department of Paediatrics and Division of clinical pharmacology and toxicology the Central laboratory Ullevål Hospital Oslo Norway) Plasma concentrations of diazepam and N-des methyldiazepam in newborn infants after intravenous intramuscular rectal and oral administration Acta Paediatr Scand 67 699 1978 —Five newborn infants (birth weight 2 900–3 600 g) were given diazepam (Valium® LaRoche) for convulsive disorders in 4 equal doses intravenously intramuscularly rectally and orally with at least 24 hours intervals Three infants were given doses of 1 mg diazepam/kg body weight and 2 0 5 mg/kg The parenteral solution of the drug was given intravenously intramuscularly and rectally Powder of tablets was given orally After intravenous administration very high peak values of plasma-diazepam concentration were obtained (5 775–10 800 ng/ml after 1 mg/kg 2 750 and 6 450 ng/ml after 0 5 mg/kg) Next to intravenous administration rectal administration caused the most rapid increase in plasma-diazepam concentration Presumed anticonsulsive concen trations (150–300 ng/ml) were obtained within 5 min with 1 mg/kg as well as 0 5 mg/kg rectally Rectal administration therefore could be a suitable treatment for seizures in the newborn infant Accumulation of the main depressive metabolite N-desmethyldiazepam oc curred in all infants This phenomenon must be taken into account when repeated doses of diazepam are *administered*

KEY WORDS Plasma-diazepam neonatal seizures rectal administration intravenous administration intramuscular administration oral administration

Treatment of neonatal convulsions will be primarily that of the causative condition to gether with supportive measures In addition anticonvulsants may be indicated and diaze pam given by intravenous injection is very effective in this respect Sometimes however technical difficulties hamper *this route of* ad ministration and alternative routes must be used In adults reports on absorption of dia zepam after intramuscular administration are conflicting (3 5 6 8 9) *while rectal* admin istration of the solution for parenteral injec tion to children causes high plasma levels of diazepam (1 7)

The purpose of the present investigation was to study the plasma levels of diazepam and the main metabolite N desmethyldiaze pam after intravenous intramuscular rectal and oral administration to newborn infants To our knowledge plasma concentrations ob tained after rectal diazepam compared to con centrations obtained by other ways of admin istration have not been reported previously in newborn infants

MATERIAL AND METHODS

Five newborn infants with birth weight 2900–3600 g ges tational age 37–40 weeks and post natal age 1–4 days were included in the series The neonates were given phenobarbital (5 mg/kg/24 hours) because of seizures Di azepam was administered either to stop recurrent convul sions or to cause sedation All the infants were given 4 equal doses of diazepam administered intravenously in tramuscularly rectally and orally with at least 24 hours intervals in a sequence which was different for each neo-

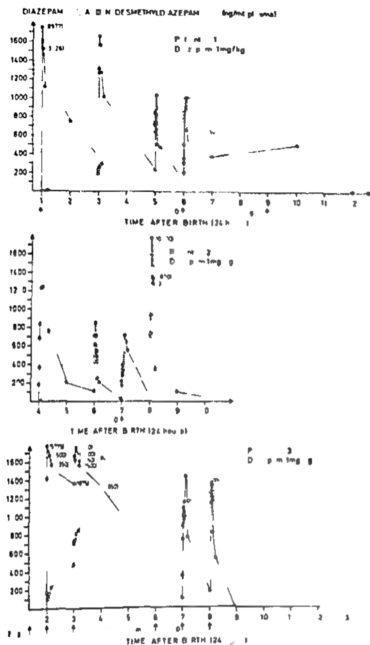


Fig 1 Time curves showing the plasma concentrations of diazepam and N-desmethyldiazepam in 3 newborn infants after repeated doses of diazepam 1 mg/kg body weight Each dose is indicated by arrow I V =intravenous I M =intramuscular R =rectal and O =oral Arrows without these symbols indicate additional diazepam administered

nate In 2 infants all the doses were 0 5 mg/kg body weight and in 3 1 mg/kg

The commercial preparation Valium® (LaRoche) for parenteral injection was used for intravenous intramuscular and rectal administration the latter by a syringe with a catheter inserted 6–8 cm into the rectum The oral doses were administered as powder of 2 mg Valium® tablets through a gastric feeding tube

Blood samples for diazepam and N desmethyldiazepam analysis were taken through an indwelling umbilical artery catheter into test tubes with heparin at 5 10 20 30 45 and 60 min and 2 4 and 24 hours after the last dose and just before the next dose In addition usually daily samples were taken for 2–7 days after the diazepam medication was stopped The blood was immediately centrifuged and the plasma stored at −20°C until analyzed

The determination of diazepam in plasma was carried out by gas liquid chromatography according to a modification of the method described by Berlin et al (2) which also allows measurement of N-desmethyldiazepam

RESULTS

All doses administered caused an increase in the plasma diazepam concentrations (Figs 1 and 2) The increase was much higher after intravenous administration than after the other doses Intramuscular oral and rectal administration gave about the same increase in plasma diazepam concentration except for intramuscular injection of 1 mg/kg which caused

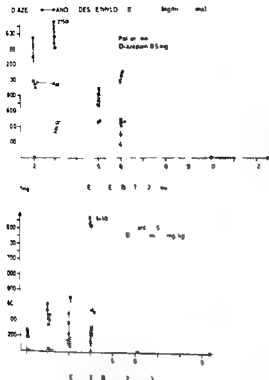


Fig 2 Time curves showing the plasma concentrations of diazepam and N-desmethyldiazepam in 2 newborn infants after repeated doses of diazepam 0 5 mg/kg body weight with symbols as in Fig 1

the plasma diazepam concentration to increase more than after rectal and oral administration (Fig 1)

After intravenous administration very high peak values (5775–10800 ng/ml after 1 mg/kg 2750 and 6450 ng/ml after 0 5 mg/kg) were obtained and always after 5 min (Figs 1 and 2) The high peaks were followed by a rapid fall in plasma diazepam within 5–15 min and than by a more slow fall

Intramuscular rectal and oral administration of diazepam in dose 1 mg/kg caused peak plasma diazepam levels after 30–60 20–30 and 60–120 min respectively (Fig 3) After 0 5 mg/kg the peak levels were obtained after 20–45 10–60 and 30–120 min respectively (Fig 4) The subsequent fall in plasma diazepam concentration after these three forms of administration was considerably slower than after intravenous injection

When the rate of the rise in plasma diazepam concentration within the first 5 and 10 min after administration is concerned the rectal administration of both 0 5 and 1 mg/kg is superior to the intramuscular administration which is superior to the oral administration (Table 1) Repeated administration of diazepam caused a gradual increase in the plasma concentration of the main metabolite N desmethyldiazepam (Figs 1 and 2) When diazepam medication was stopped the plasma concentration of the metabolite was usually higher than of diazepam and was always higher 24 hours after termination of the medication and remained so for the 2–7 days sampling was continued (Figs 1 and 2)

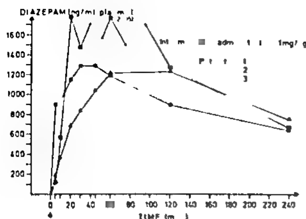
Administration of diazepam intravenously intramuscularly and rectally (0 5 as well as 1 mg/kg) arrested seizures Oral medication was only given for sedation but seemed effective in this respect

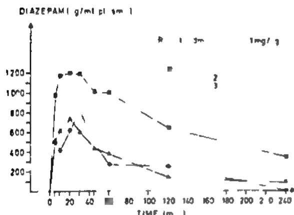
In 2 patients considerable muscular hypotonia was observed several days after the diazepam medication was stopped Respiratory depression or any other side effects attributable to the diazepam medication were not observed

Table 1 *Mean rate of rise of plasma diazepam concentrations in newborn infants within the first 5 and 10 min after administration of diazepam*

Three infants were given 1 mg diazepam/kg and 2 were given 0 5 mg/kg

Route of administration	Dose (mg/kg)	Increase in plasma-diazepam concentration (ng/ml/min)	
		In 5 min	In 10 min
Rectally	1	133	74
Intramuscularly	1	80	67
Orally	1	30	27
Rectally	0 5	66	41
Intramuscularly	0 5	48	37
Orally	0 5	13	17





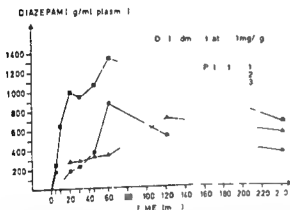
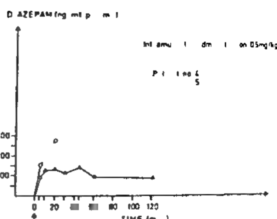
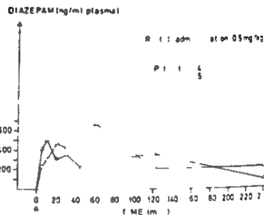


Fig 3 Time curves showing the plasma concentrations of diazepam in 3 newborn infants after intramuscular, rectal and oral administration of diazepam in dose 1 mg/kg body weight. The curves have been constructed from the diazepam values shown in Fig 1 by subtracting the plasmadiazepam concentrations present just before administration of diazepam, and transferring the curves in parallel to the same starting point.





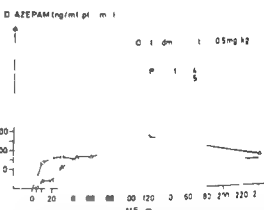


Fig 4 Time curves showing the plasma concentrations of diazepam in 2 newborn infants after intramuscular, rectal and oral administration of diazepam in dose 0.5 mg/kg body weight. The curves have been constructed from the diazepam values shown in Fig 2 by subtracting the plasma diazepam concentrations present just before administration of diazepam, and transferring the curves in parallel to the same starting point.

DISCUSSION

In the present investigation diazepam solution administered by rectum was more rapidly absorbed than by intramuscular injection and more rapidly after intramuscular injection than by oral administration. Because of the rapid increase in plasma diazepam concentration after rectal administration, which is in accordance with findings in older children (1, 7), we find this way of administration to be the best al-

ternative to the intravenous route in treating ongoing seizures in newborn infants

The anticonvulsive range of plasma-diazepam concentration has hitherto not been established with certainty although some observations in children lend support to 150–300 ng/ml as threshold for seizure recurrence when the plasma concentration is falling (1 4) Values far above this range were obtained within 5 min with intravenously administered diazepam (0 5 as well as 1 mg/kg) and also with rectally administered diazepam in dose 1 mg/kg A dose of 0 5 mg diazepam/kg gave plasma concentrations in the threshold area In spite of clinical effect on seizures with this dose it seems reasonable to administer about 1 mg/kg of diazepam rectally if the drug is given to stop ongoing convulsions in newborn infants

The intravenously administered doses in the present study were too high as judged from the very high peak values obtained The high plasma diazepam concentrations however seemed to be well tolerated Plasma diazepam falls very steeply after intravenous administration with the risk of recurrence of convulsions It is possible that the combination of a smaller intravenous dose to rapidly stop ongoing seizures and a rectal (or intramuscular) dose would give the most suitable profile of plasma diazepam also maintaining anticonvulsive levels for a longer time

The oral dosage form is not suitable for treatment of acute seizures In situations where rapid absorption is not mandatory however the drug is sufficiently absorbed after oral administration to be effective for sedation in newborn infants

Some investigators have found a very unreliable absorption of intramuscular diazepam to adults (5 6 9) while others have found the drug to be rapidly absorbed (3 8) In the present study intramuscularly administered diazepam was rapidly absorbed The injections were given laterally in the thigh and the injection site rubbed gently for 1–2 minutes after the injection This procedure as well as age related changes in the tissues may probably influence the absorption of diazepam from muscles

In the neonatal period diazepam and N desmethyldiazepam have longer plasma half life than in older children and adults (11) The half life of the metabolite is much longer than the parent compound (11) This phenomenon can cause accumulation of the metabolite , as shown from our series The plasma concentrations of N desmethyldiazepam were of the magnitude which we have previously observed in association with respiratory and muscular depressive effects on neonates (10) In our series two infants had severe muscular hypotonia most likely caused by N desmethyldiazepam One therefore should take the possibility of accumulation of the depressive metabolite N desmethyldiazepam into account when repeated doses of diazepam are administered to newborn infants

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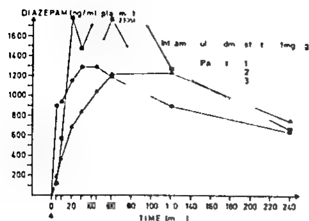
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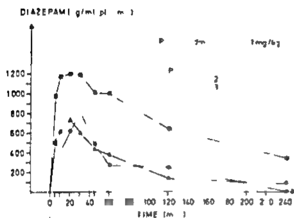
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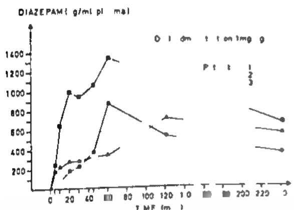
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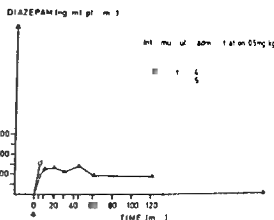
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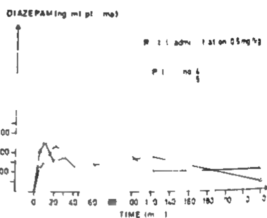
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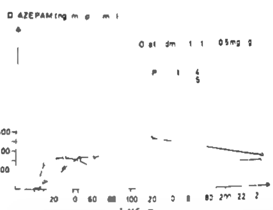


Fig 3 Time curves showing the plasma concentrations of diazepam in 3 newborn infants after intramuscular rectal and oral administration of diazepam in dose 1 mg/kg body weight The curves have been constructed from the diazepam values shown in Fig 1 by subtracting the plasmadiazepam concentrations present just before administration of diazepam and transferring the curves in parallel to the same starting point

Fig 4 Time curves showing the plasma concentrations of diazepam in 2 newborn infants after intramuscular rectal and oral administration of diazepam in dose 0 5 mg/kg body weight The curves have been constructed from the diazepam values shown in Fig 2 by subtracting the plasma diazepam concentrations present just before administration of diazepam and transferring the curves in parallel to the same starting point

DISCUSSION

In the present investigation diazepam solution administered by rectum was more rapidly absorbed than by intramuscular injection and more rapidly after intramuscular injection than by oral administration Because of the rapid increase in plasma diazepam concentration after rectal administration which is in accordance with findings in older children (1 7) we find this way of administration to be the best al

ternative to the intravenous route in treating ongoing seizures in newborn infants

The anticonvulsive range of plasma-diazepam concentration has hitherto not been established with certainty although some observations in children lend support to 150–300 ng/ml as threshold for seizure recurrence when the plasma concentration is falling (1 4). Values far above this range were obtained within 5 min with intravenously administered diazepam (0 5 as well as 1 mg/kg) and also with rectally administered diazepam in dose 1 mg/kg A dose of 0 5 mg diazepam/kg gave plasma concentrations in the threshold area In spite of clinical effect on seizures with this dose it seems reasonable to administer about 1 mg/kg of diazepam rectally if the drug is given to stop ongoing convulsions in newborn infants

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IMMUNODEFICIENCY IN DOWN S SYNDROME

Titres of Natural Antibodies to E coli and Rabbit Erythrocytes at Different Ages

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ABSTRACT Ugazio A G Lanzavecchia A Jayakar S Plebani A Duse M and Burgio G R (Department of Paediatrics Department of Dermatology and the CNR Laboratory for Biochemical and Evolutionary Genetics University of Pavia Pavia Italy) Immunodeficiency in Down s syndrome titres of natural antibodies to E coli and rabbit erythrocytes at different ages Acta Paediatr Scand 67 705 1978.— Natural antibody titres to *E coli* O antigens of different serotypes and to rabbit red blood cells were determined in 86 subjects with Down s syndrome and 79 mentally retarded but chromosomally normal controls ranging in age from 10 months to 52 years Subjects in the two groups were matched for sex age and socio-environmental conditions Titres of both antibodies assessed by haemagglutination were significantly lower in subjects with DS in the 1 to 5 year old group *E coli* antibodies transiently increased to normal values in subjects with DS during the second 5 years of life thereafter rapidly declining to levels significantly lower than those observed in controls The titres of antibodies to rabbit erythrocytes in subjects with Down s syndrome showed a more variable course transiently approaching normal values in the 7–10 year group and after 20 years of age These data are interpreted as further evidence for the existence of a congenital immunodeficiency in Down s syndrome

KEY WORDS Down s syndrome immunodeficiency natural antibodies

Growing clinical and laboratory evidence suggests that immunodeficiency is an integral feature of Down s syndrome (DS) (3 9) In a recent longitudinal survey mortality for respiratory and extra respiratory infections has been reported to be respectively 124 and 52 times higher in DS than in the general population (7) Furthermore there is now firm laboratory evidence that subjects with DS have a deficiency of both cell and antibody mediated immunity (2 5 10 15) Whether this immunodeficiency is congenital (primary) or acquired as a result of preferential exposure to unfavorable environmental conditions (institutionalization) remains the object of considerable theoretical and experimental debate (3 9 15)

Whittingham et al (15) recently reported that young adult subjects with DS have low titres of natural antibodies to flagellin and this finding is interpreted by the authors as the results of a stress deficiency of the immune system resulting from repeated infections probably attributable to rearing under unfavorable environmental conditions

To test this hypothesis we measured titres of natural antibodies against different serotypes of *E coli* and against rabbit erythrocytes (RaRBC) in subjects with DS at different ages and in controls matched for age sex and environmental conditions

MATERIAL AND METHODS

Test subjects

86 patients with DS and 79 control subjects of age ranging from 10 months to 52 years were included in this study All subjects over the age of 13 were from one of three large institutions and were matched with chromosomally normal

Table 1 *Antibodies to E coli and Rabbit Erythrocytes (RaRBC) in patients with Down's Syndrome (DS) and Controls (C) in different age groups mean log titres and significance of the difference*

Age group (years)	Number of subjects		*E coli* antibodies (Mean log_2 titre)			RaRBC antibodies (Mean log_2 titre)		
	DS	C	DS	C	Significance	DS	C	Significance
1–2	11	10	3.82	6.00	$p<0.001$	[illegible]36	8.30	$p<0.05$
2–5	9	8	4.56	7.13	$p<0.001$	7.78	9.13	$p<0.0007$
5–7	7	7	5.71	6.71	N S	7.71	10.43	$p<0.001$
7–10	12	10	6.08	6.70	N S	7.83	9.50	N S
10–20	20	16	5.35	7.63	$p<0.0002$	7.80	9.38	$p<0.005$
20–30	11	14	5.64	7.29	$p<0.01$	8.45	9.50	N S
>30	16	14	5.25	6.43	$p<0.005$	7.13	7.86	N S

Wilcoxon rank test N S not significant

mentally defective subjects from the same institution Subjects with DS who were less than 13 years of age were not institutionalized those attending school were age and sex matched with mentally retarded children from the same school those not attending school were age and sex matched with mentally retarded children living at home under similar socio-environmental conditions All the subjects with DS were primary trisomics controls had normal karyotypes and those with chromosomal abnormalities detected by chance during the examination were excluded from the study

All subjects were in good general health and were taking no drugs at the time of the study

Blood was obtained by venipuncture and allowed to clot sera were stored at −30 C until tested

Sera

Sera were heat inactivated at 56 C for 30 minutes and centrifuged at 5000 rpm As a control an aliquot was incubated with an equal volume of phosphate buffered saline pH 7.3 (PBS) containing 0.1 mol/l 2 mercapto ethanol for 30 minutes at 37 C After this treatment the agglutinating titres against both *E coli* and rabbit erythrocytes decreased sharply in all the samples tested

Absorption of the sera with 10% (V/V) of packed RaRBC (1 hr at room temperature with occasional shaking) virtually abolished the agglutinating titre against RaRBC while the titre of anti *E coli* antibodies remained unchanged

E coli antibodies

Antibodies against *E coli* O antigens of six different serotypes (01 K1 H7 02 K1 H4 04 K3 H5 06 K?ac H1 015 K14 H4 075 K?H5) were assayed by passive haemagglutination as described by Webster (14)

In brief 100 µl of packed group O human red blood cells were sensitized with 1 ml of a pool of the six different antigens at 37 C for 20 minutes washed and resuspended at 1% Twofold dilution of the sera to be tested were set up with PBS containing 0.1% bovine serum albumin (BSA) in 50 µl volumes in U bottomed microtitre plates 50 µl of sensitized red cells were then added and agglutination read after 2 hours of incubation at 37 C Controls with unsensitized human red blood cells were always negative

Antibodies to rabbit erythrocytes

Sera were diluted in PBS containing 0.1% BSA and 50 µl of a 0.75% suspension of washed RaRBC added Plates were incubated at 4 C overnight

Statistical evaluation

In order to smooth the variation of antibody titres with age the subjects of each group were arranged according to age and then 10-point moving averages both of age and of log_2 titre were computed for sets of 10 consecutive subjects and each point was plotted on the graph

In order to compare the differences between DS and controls they were divided on the basis of age into roughly equal groups and within each age group a two-tailed Wilcoxon Rank test was used (Table 1)

RESULTS

Titres of antibodies to *E coli* in the various age groups of subjects with DS and controls are reported in Fig 1 As shown in Table 1 the titre of *E coli* antibodies was significantly lower in patients with DS as compared with controls in all age groups except the 5–10 year groups

RaRBC antibody titres were significantly lower in DS than in controls up to the 7th year from 7 to 10 years the difference was not significant but thereafter titres were again significantly lower in DS up to the age of 20 After 20 years the difference was no longer significant (Fig 2)

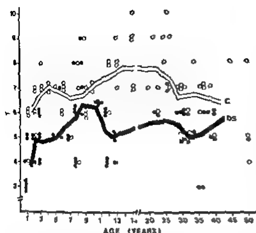


Fig. 1. Titres of *E. coli* antibodies in subjects with Down's Syndrome (DS) (●) and in matched controls (C) (O) of age ranging from 10 months to 57 years. The moving averages (see methods) of the titres for both DS (▬) and controls (═) are also reported.

DISCUSSION

The present data show that during the first years of life "natural" antibody titres are definitely lower in children with DS than in controls.

It is generally agreed that "natural" antibodies, mostly IgM, result from exposure to widespread antigenic stimuli (11). Since it is very unlikely that subjects with DS are less exposed to these antigens than are controls, it seems reasonable to postulate that the difference lies in the patterns of immune response.

It has been claimed that immunodeficiency in DS is the result of repeated infections leading to "exhaustion" of the immune system; this has been attributed to rearing under unfavorable environmental conditions related either to institutionalization or to an incapacity to maintain adequate standards of personal hygiene (15). In the present study, both children with DS and mentally retarded controls under 13 years of age were cared for at home by their families; the finding of low antibody titres in this group of young children with DS can reasonably be taken as evidence against both these hypotheses. Furthermore, recent studies have demonstrated that infants and children with DS also have a significantly reduced percentage of circulating T lymphocytes (5, 12) as well as an increased frequency of thyroglobulin antibodies (13). All these data suggest that immunodeficiency is a congenital (primary) feature of DS.

As for the time course observed for "natural" antibody titres, it is evident that the development of normal serum concentrations of these antibodies is somewhat delayed in subjects with DS, temporarily reaching control values only between 5 and 10 years of age. These results are helpful in interpreting the previous, apparently contradictory observations of Adinolfi (1), who reported normal isohaemagglutinin titres in children with DS from 4 to 16 years old (most of them under 10) and of Whittingham (15), who reported low levels of "natural" antibodies in adults with DS. The cellular and molecular basis of this delayed maturation in subjects with DS remains to be explained. Since there is fairly good evidence that a deficiency of the thymus dependent system is a very early event in DS (5, 9, 12) it is tempting to speculate that "natural" antibody responses are normally more thymus dependent during the first few years than at later

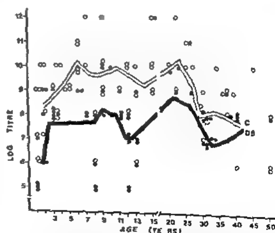


Fig. 2. Titres of antibodies to rabbit erythrocytes in subjects with Down's syndrome (DS) (●) and in matched controls (C) (O) of age ranging from 10 months to 57 years. The moving averages (see Methods) of the titres for both DS (▬) and controls (═) are also reported.

ages The low titres of natural antibodies especially against *E coli* observed in the older age groups are likely to be the result of a different immune mechanism i e precocious ageing of the immune system in DS

It has been proposed recently that a selective T cell defect could be the primary event in DS leading to disturbed immune homeostasis (3) and consequently to increased susceptibility to infections (7) appearance of autoantibodies (13) and increased risk of malignancies (6) from the first few years of life Repeated infections which are known to depress the immune function (4 8) can in turn stress the already unbalanced immune system leading to precocious ageing of both cell mediated and antibody mediated immunity

On these grounds the course of natural antibody titres against these unrelated antigens observed here might reflect the peculiar evolution of this immunodeficiency low titres in children with DS could be the result of the primary T cell deficiency and low titres in young adults with DS could be the result of precocious ageing of the whole immune system

ACKNOWLEDGEMENTS

We thank Mr A Ascione for expert technical assistance and Dr A D B Webster Clinical Research Centre Watford Road Harrow Middlesex for kindly supplying the *E coli* antigens This work was partly supported by CNR Rome

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POSSIBLE EFFECTS OF KANAMYCIN AND INCUBATION IN NEWBORN CHILDREN WITH LOW BIRTH WEIGHT

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ABSTRACT Winkel S Bonding P Kildegård Larsen P and Roosen J (Department of Neonatal Pediatrics and the Ear Nose and Throat Department University Hospital of Copenhagen Tagensvej III Copenhagen Denmark) Effects of kanamycin and incubation in newborn children with low birth weight Acta Paediatr Scand 67 709 1978.—In an acoustico-vestibular follow up investigation of 91 four to six year-old children with birth weight below 2000 g the same incidence of sensorineural hearing loss (19%) was found in 54 children treated with kanamycin in the neonatal period as in a group of 37 infants not treated with kanamycin When comparing a group of children treated with both kanamycin and incubator (54 children) with a group treated with incubator only (16 children) no definite signs of synergism between incubator noise and kanamycin were found However the 5 cases of moderate or severe hearing loss all belonged to the group treated with both incubator and kanamycin These 5 children had more severe neonatal complications especially apnea cyanotic spells and hyperbilirubinemia which may increase the severity of the hearing loss

Among 56 incubator treated children with normal hearing (ISO standards) 57% had an audiogram pattern suggesting minor noise provoked cochlear lesions Among 18 non incubator treated children with normal hearing only one child (6%) had a similar pattern It should be stressed however that these children had no clinical symptoms of hearing loss

KEY WORDS Newborn low birth weight kanamycin incubator noise hearing loss

The good clinical effect of kanamycin (Km) on infections in the neonatal period has resulted in widespread use of this antibiotic Similar to other aminoglycosides KM is ototoxic and may cause irreversible damage to the hair cells of the cochlea However when treating neonatal infections with KM in a dose of 10–15 mg/kg/24 h for 5–10 days no ototoxic effect was observed according to follow up investigations of 4 to 6-year old children by Eichenwald (7) Fujii (9) and Sanders et al (12)

Premature infants are more often exposed to conditions which on their own can cause damage of the cochlea and the central hearing area especially asphyxia and hyperbilirubinaemia (9)

Furthermore the premature infant is often treated in an incubator Animal studies by Dayal et al (6) have shown a synergistic effect of KM and incubator noise Recently incubator noise has been suggested (4 8) as a possible contributory cause of deafness in children As a consequence the purpose of this work has been to investigate whether (*a*) after neonatal treatment with a standard dose of KM premature children develop sensorineural hearing loss which can be related to treatment with KM (*b*) there are signs of synergistic effect between KM and incubator treatment and (*c*) premature infants develop noise provoked lesions after treatment with incubator

MATERIAL AND METHODS

The material comprises children admitted to the neonatal department of Rigshospitalet during the period 1967–69 all having a birth weight ≤2000 g Excluded from the investigation are children who have been treated with other potentially ototoxic drugs in particular streptomycin Of the 148 children who fulfilled these criteria 95 children appeared at the follow up examination In 4 children audiometry could not be performed due to lack of cooperation Thus the follow up was performed in 91

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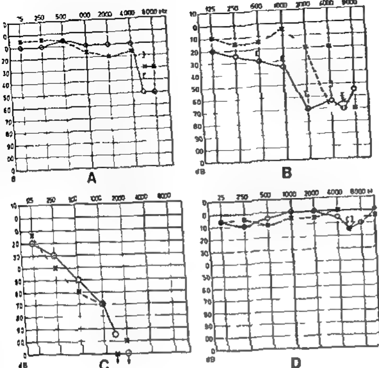


Fig 1 Examples of sensorineural hearing loss and of noise dip A Slight B Moderate C Severe D Noise dip

group treated with KM + incubator (group 1) with the group treated with incubator only (group 2) the latter group showed a slightly but not significantly higher incidence of hearing loss (25%) In the group of children treated with neither incubator nor KM (group 3) the incidence of sensorineural hearing loss was 14% This difference is not significant

When comparing neonatal complications and treatment in the group with and without sensorineural hearing loss (Table 3) a significantly higher incidence of late asphyxia ($p<0\ 002$) was found in the group with hearing loss The incidence of hyperbilirubinaemia was higher—but not significant ($p=0\ 07$) in the group with hearing loss

Among the other parameters no significant differences were found In particular nearly the same percentage of children in the two groups was treated with incubator and with KM

Detailed information about the 5 children with moderate or severe sensorineural hearing loss is given in Table 4 It is seen that all but

Table 2 *Incidence and severity of sensorineural hearing loss*

Group 1 + Kanamycin + Incubator Group 2 − Kanamycin + Incubator Group 3 − Kanamycin − Incubator

	Group 1		Group 2		Group 3		Groups 1+2+3	
	n	%	*n*	%	*n*	%	*n*	%
No in group	54	100	16	100	21	100	91	100
Slight hearing loss	5	9	4	25	3	14	12	[illegible]
Moderate hearing loss	3	6	0	0	0	[illegible]	3	3
Severe hearing loss		4	0	0	0	0	[illegible]	2
Total with hearing loss			4	5	3	14		
	10	19		7	=19%		17	[illegible]

Table I *Birth weight and neonatal complications in the total material*

Group 1 + Kanamycin + Incubator Group 2 − Kanamycin + Incubator Group 3 − Kanamycin − Incubator

	Group 1		Group 2		Group 3	
	n	%	*n*	%	*n*	%
Total number	54	100	16	100	21	100
Bright weight						
≤1 000 g	3	6	0	0	0	0
1 001–1 500 g	21	39	5	31	2	10
1 501–2 000 g	30	55	11	69	19	90
Birth asphyxia (Apgar score <7 1–5 min)	23	43	2	13	1	5
Late asphyxia (apnea cyanosis)	31	57	6	38	4	19
Hyperbilirubinaemia (bilirubin >10 mg/100 ml)	35	65	12	75	13	62
Exchange transfusion	17	31	7	44	3	14

(61%) of the 140 children summoned At the time of the follow up investigation the children were aged 4–6 years

The children were divided into three groups Group 1 had been treated with KM as well as incubator (54 children) group 2 had not been treated with KM but had been in an incubator (16 children) and group 3 had been treated with neither KM nor incubator (21 children)

The KM treatment was given either prophylactically or as a part of the treatment against infections during the neonatal period The dose was 10 mg/kg/24 h divided into two doses and lasted 5 days or longer Serum concentrations were not measured The incubator treatment lasted more than 12 hours in all cases Two different types of incubators were used—Ohio and Airshield The noise level in these incubators corresponded to the noise level found in other more extensive studies on this subject e g Blennow et al (7) and Falk & Farmer (8) who found a noise level of 58–75 dB (A)

For many of the children the neonatal period was complicated with a number of those conditions that usually threaten the premature infant The three groups of the children are not completely comparable since group 1 which was treated with KM as well as incubator more often presented the most serious problems especially asphyxia and very low birth weight (Table 1)

Otological and developmental history were obtained previous to the investigation through a distributed questionnaire Information about the neonatal period was obtained by studying the charts

The examination consisted of a general otological examination and an acoustico vestibular examination including calorimetry *ad modum* Hallpike Audiometry was performed by two experienced audiometry assistants using a Madsen electronic audiometer OB 70 in a sound proof chamber

Sensorineural hearing loss in this investigation was defined (according to ISO R 389 (1967)) as a higher air conduction threshold than 20 dB (HL) for at least one frequency In the case of conductive hearing loss (mostly secretory otitis media) this was eliminated before the actual evaluation In a few cases of conductive hearing loss the bone conduction threshold was used as a parameter instead of the air conduction threshold

Sensorineural hearing loss was graduated as follows (Fig 1 A–D)

Slight normal hearing in the conversation area (500–4 000 Hz) For frequencies above 4 000 Hz a higher threshold than 20 dB (HL) for at least one frequency

Severe affected social hearing hearing aid needed Threshold bilaterally higher than 30 dB (HL) in the conversation area

Moderate remaining cases

Besides sensorineural hearing loss special interest was paid to possible noise provoked minor lesions noise dips among children with normal hearing according to the definitions above

Noise dip A selective higher threshold of ≥10 dB (HL) in the range 4–6 000 Hz compared with the threshold of the surrounding frequencies in patients without sensorineural hearing loss

Student's *t* test was used for the statistical calculations

RESULTS

Sensorineural hearing loss was found in 17 children (19%) as shown in Table 2 In all cases the impairment of hearing was typically a high tone loss Moderate or severe hearing loss was found in 5 of the 10 children with hearing loss in the KM group (group 1) while all cases of hearing loss in the non KM groups (groups 2+3) were classified as slight

According to Table 2 the same incidence of sensorineural hearing loss (19%) was found in the KM group (group 1) as in the non KM groups (groups 2+3) When comparing the

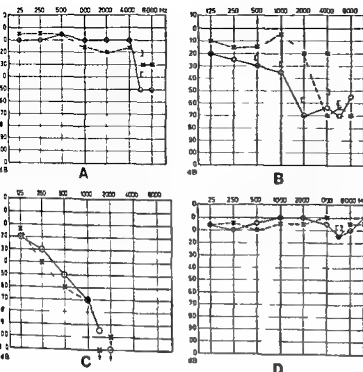


Fig 1 Examples of sensorineural hearing loss and of noise dip A Slight B Moderate C Severe D Noise dip

group treated with KM + incubator (group 1) with the group treated with incubator only (group 2) the latter group showed a slightly but not significantly higher incidence of hearing loss (25%) In the group of children treated with neither incubator nor KM (group 3) the incidence of sensorineural hearing loss was 14% This difference is not significant

When comparing neonatal complications and treatment in the group with and without sensorineural hearing loss (Table 3) a significantly higher incidence of late asphyxia ($p<0.002$) was found in the group with hearing loss The incidence of hyperbilirubinaemia was higher—but not significant ($p=0.07$) in the group with hearing loss

Among the other parameters no significant differences were found In particular nearly the same percentage of children in the two groups was treated with incubator and with KM

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Group 1 + Kanamycin + Incubator Group − Kanamycin + Incubator Group 3 − Kanamycin − Incubator

	Group 1		Group 2		Group 3		Groups 1+2+3	
	n	%	*n*	%	*n*	%	*n*	%
No in group	54	100	16	100	21	100	91	100
Slight hearing loss	5	9	4	25	3	14	[illegible]	[illegible]
Moderate hearing loss	3	[illegible]	0	0	0	0	3	3
Severe hearing loss		4	0	0	[illegible]	0	[illegible]	2
Total with hearing loss	10	19	4	25	3	14	17	19
			7	=19% (Groups 2+3)				

Table 3 *Neonatal complications and treatment in children with and without sensorineural hearing loss*

	With sensorineural hearing loss		Without sensorineural hearing loss		
	n	%	n	%	Significance
No in group	17	100	74	100	
Birth asphyxia (Apgar ≤7 1–5 min)	5	29	21	28	n s
Late asphyxia (cyanosis apnea)	14	82	27	36	$p=<0\ 002$
Hyperbilirubinaemia	15	88	45	61	n s
Exchange transfusion	6	35	21	28	n s
KM treatment	10	59	44	59	n s
Incubator	14	82	56	76	n s

one child had experienced some degree of late asphyxia and that all but one had severe hyperbilirubinaemia when their weight is considered

Noise dip Among 56 incubator treated children (groups 1+2) without sensorineural hearing loss 29 children (52%) (Table 5) had an audiogram pattern suggesting minimal cochlear lesions similar to noise provoked lesions Among 18 non incubator treated children (group 3) without sensorineural hearing loss only one child (6%) had a similar pattern The difference is significant ($p<0\ 002$)

No sign of synergistic effect between KM and incubator noise was found The percentage of noise dip (48%) in the group treated with both KM and incubator (group 1) was slightly lower than in the group treated with incubator only (67%) (Group 2)

Comparing neonatal complications and treatment in the groups of children with and without noise dip (Table 6) the only significant difference ($p<0\ 002$) was that among the group with noise dip a higher percentage (97%) were incubator treated than those without (61%) Furthermore it should be mentioned that there was no relationship between the duration of the incubator treatment and the presence of noise dip the incidence was the same among children treated for 1–7 days 8–14 days and more than 21 days

Abnormal vestibular findings were found in only one out of all 91 examined children This child also had a moderate sensorineural hearing loss It should be noted however that it

Table 4 *Detailed information about the 5 children with moderate or severe sensorineural hearing loss*

Child	1	2	3	4	5
Degree of hearing loss	Severe	Severe	Moderate	Moderate	Moderate
Birth Weight	1 300 g	1 550 g	1 980 g	1 750 g	1 250 g
Birth asphyxia	Slight	No	Slight	No	No
Late asphyxia (apnea cyanosis)	No	Yes	Yes	Yes	Yes
Hyperbilirubinaemia (mg/100 ml)	Max 17 6	Max 22 3	Max 17	Max 11 8	Max 20 6
Exchange transfusion	Yes	No	No	No	4 times
KM duration	6 days	5 days	6 days	5 days	5 days
Incubator duration	18 days	8 days	11 days	13 days	13 days
Respirator treatment	No	No	No	No	No
Middle ear infections	No	Several	Several	No	No
Signs of cerebral damage	Cerebral palsy athetosis	No	No	No	Delayed psychomotor development

Table 5 *Incidence of noise dip in children without sensorineural hearing loss*
Group 1 Kanamycin + Incubator Group 2 − Kanamycin + Incubator Group 3 − Kanamycin − Incubator

	Group 1		Group		Group 3		Group 1+2+3	
	n	%	*n*	%	*n*	%	*n*	
Number in group	44	100	1	100	[illegible]	100	74	100
Noise dip	21	48	8	67	1	[illegible]	30	41
		9		5 %				

was difficult to obtain a conclusive result in several of the children especially in the calorimetric examination

DISCUSSION AND CONCLUSIONS

Evaluation of the results is complicated by the relatively low percentage (61%) in the follow up In spite of repeated attempts it has not been possible to improve the follow up percentage In order to exclude that the remaining 53 children who did not appear at the follow up examination had severe hearing defects inquiries were sent to all audiological centres in Denmark It was hereby established that none of the remaining children had applied for audiological assistance and so are not likely to have symptoms of hearing reduction

Audiometry is a procedure which requires active cooperation and it is therefore charged with some uncertainty The age group chosen for the follow up investigation however is usually a group in which the hearing threshold is stated fairly accurately although higher than for adults 20 dB (HL) is the limit for normality However the results would not differ considerably when choosing 30 dB (HL) as 15 of the 17 cases of hearing loss had threshold higher than 30 dB for at least one frequency

Sensorineural hearing loss

There was no obvious synergistic effect of KM and incubator noise The group treated with both incubator and KM (group 1) had a slightly lower (but not significant) percentage of hearing loss (19%) compared with the group treated with incubator only (group 2) (25%)

However all 5 cases of moderate or severe hearing loss were found in the group treated with both KM and incubator (group 1) This could point to the assumption that KM sensitized the inner ear to other trauma such as asphyxia hyperbilirubinaemia and noise and thereby exaggerated its effect on the cochlea This possibility cannot be excluded

Treatment with standard doses of KM without knowing serum concentration complicates

Table 6 *Neonatal complications and treatment in children without sensorineural hearing loss with and without noise dip*

	With noise dip		Without noise dip		
	n	%	*n*	%	Significance
No in group	30	100	44	100	
Birth asphyxia (Apgar ≥7 1 5 min)	8	7	13	30	n s
Late asphyxia (cyanosis-apnea)	14	47	13	30	n s
Hyperbilirubinaemia	19	63	6	59	n s
Exchange transfusion	10	33	11	5	n s
Incubator	29	97	27	61	$p = < 0\ 00$
KM	1	70	23	52	n s

Table 3. *Neonatal complications and treatment in children with and without sensorineural hearing loss*

	With sensorineural hearing loss		Without sensorineural hearing loss		
	n	%	n	%	Significance
No. in group	17	100	74	100	
Birth asphyxia (Apgar ≤7, 1–5 min)	5	29	21	28	n.s.
Late asphyxia (cyanosis, apnea)	14	82	27	36	$p=<0.002$
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one child had experienced some degree of late asphyxia and that all but one had severe hyperbilirubinemia when their weight is considered.

Noise dip. Among 56 incubator treated children (groups 1+2) without sensorineural hearing loss, 29 children (52%) (Table 5) had an audiogram pattern suggesting minimal cochlear lesions similar to noise provoked lesions. Among 18 non-incubator treated children (group 3) without sensorineural hearing loss only one child (6%) had a similar pattern. The difference is significant ($p<0.002$).

No sign of synergistic effect between KM and incubator noise was found. The percentage of noise dip (48%) in the group treated with both KM and incubator (group 1) was slightly lower than in the group treated with incubator only (67%) (Group 2).

Comparing neonatal complications and treatment in the groups of children with and without noise dip (Table 6), the only significant difference ($p<0.002$) was that among the group with noise dip a higher percentage (97%) were incubator treated than those without (61%). Furthermore, it should be mentioned that there was no relationship between the duration of the incubator treatment and the presence of noise dip: the incidence was the same among children treated for 1–7 days, 8–14 days and more than 21 days.

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Table 4. *Detailed information about the 5 children with moderate or severe sensorineural hearing loss*

Child	1	2	3	4	5
Degree of hearing loss	Severe	Severe	Moderate	Moderate	Moderate
Birth Weight	1 300 g	1 550 g	1 980 g	1 250 g	1 250 g
Birth asphyxia	Slight	No	Slight	No	No
Late asphyxia (apnea, cyanosis)	No	Yes	Yes	Yes	Yes
Hyperbilirubinaemia (mg/100 ml)	Max. 17.6	Max. 22.3	Max. 17	Max. 11.8	Max. 20.6
Exchange transfusion	Yes	No	No	No	4 times
KM duration	6 days	5 days	6 days	5 days	5 days
Incubator duration	18 days	8 days	11 days	13 days	13 days
Respirator treatment	No	No	No	No	No
Middle ear infections	No	Several	Several	No	No
Signs of cerebral damage	Cerebral palsy athetosis	No	No	No	Delayed psychomotor development

Table 5 *Incidence of noise dip in children without sensorineural hearing loss*

Group I Kanamycin + Incubator Group – Kanamycin + Incubator Group 3 – Kanamycin – Incubator

	Group I		Group		Group 3		Group 1+ +3	
	n	%	*n*	%	*n*	%	*n*	
Number in group	44	100	1	100	18	100	74	100
Noise dip	21	48	8	67	1	6	30	41
		29	=	52%				

was difficult to obtain a conclusive result in several of the children especially in the calorimetric examination

DISCUSSION AND CONCLUSIONS

Evaluation of the results is complicated by the relatively low percentage (61%) in the follow up In spite of repeated attempts it has not been possible to improve the follow up percentage In order to exclude that the remaining 53 children who did not appear at the follow up examination had severe hearing defects inquiries were sent to all audiological centres in Denmark It was hereby established that none of the remaining children had applied for audiological assistance and so are not likely to have symptoms of hearing reduction

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	With noise dip		Without noise dip		Significance
	n	%	*n*	%	
No in group	30	100	44	100	
Birth asphyxia (Apgar ≥7 1–5 min)	8	7	13	30	n s
Late asphyxia (cyanosis apnea)	14	47	13	30	n s
Hyperbilirubinaemia	19	63	6	59	n s
Exchange transfusion	10	33	11	25	n s
Incubator	9	97		61	$p = < 0\ 00$
KM	1	70	23	5	n s

the evaluation We cannot exclude that the 5 children with moderate or severe hearing loss who all belonged to the group treated with KM and incubator (group 1) were the children with the highest serum levels However among these 5 children 2 suffered from obvious brain damage (athetosis retarded psychomotor development) (Table 4)

The study does not allow us to conclude which factors were responsible for the hearing loss in the 17 children Most likely it is multifactorial but by looking at the neonatal complications in the group with and without hearing loss (Table 3) it is evident that a substantial number of children with hearing loss had experienced late asphyxia (apnea or cyanosis after birth) and hyperbilirubinaemia However only occurrence of late asphyxia was found to be significant

Incubator noise alone could hardly have caused the sensorineural hearing loss since noise provoked hearing loss is typically found in the audiogram as noise dip in the area of 4 000 Hz

The incidence of hearing loss among premature infants is in accordance with the results of Campanelli et al (3) who among 44 premature children (below 2 500 g) found 16% with an average threshold of 50 dB (HL) at 1 000 Hz i e severe hearing loss

Noise dip

The American Academy of Pediatrics Committee on Environmental Hazards (1) has emphasized that newborn infants in incubator are exposed to continuous noise for days or weeks and may be more sensitive to noise In animal experiments Dayal et al (6) found only insignificant traumatic effects of protracted exposure to incubator noise alone Recently however Douek et al (4) found significant traumatic effects of incubator noise alone applied to newborn guinea pigs Other animal experiments have clearly shown synergistic effects of noise and KM not only from intense exposure to noise and large doses of KM (5 11) but also by applying incubator noise and therapeutic KM doses for 5 weeks (6)

Thirty children all but one incubator treated and belonging to the group without sensorineural hearing loss had an audiogram pattern suggesting minimum noise provoked cochlear damage

Even though a threshold of 15–20 dB (HL) in the audiogram may be a normal finding the location in the area 4–6 000 Hz which is typical for noise provoked lesions points firmly to the assumption that incubator noise was responsible

In all cases the noise dips were only minor and without importance for the actual hearing However whether such a minimal cochlear damage will become important for the children later in life for example by increasing the risk of severe noise lesions by later exposure to noise is unclarified but the possibility cannot be excluded

The intensity of the noise in incubators is described in previous works by Blennow et al (2) and Falk & Farmer (8) as 58–75 dB (A) The recorded noise levels must be considered low (normal speech corresponds to 50–60 dB (A) and furthermore are concentrated in the low frequency area where the sensitivity to noise is lowest

Nevertheless since incubator noise seems to be the cause of the recorded noise dip it is reasonable to recommend strongly the development of more silent incubator models as well as to insure careful maintenance of fans in the incubators

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ALLERGY OTITIS MEDIA AND SERUM IMMUNOGLOBULINS AFTER ADENOIDECTOMY

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ABSTRACT Kjellman N I M Harder H Hansson L O and Lindwall L (The Departments of Paediatrics Otorhinolaryngology Clinical Chemistry and Mathematics Linkoping University Linkoping Sweden) Allergy otitis media and serum immunoglobulins after adenoidectomy Acta Paediatr Scand 67 717 1978.—The incidence of atopic disease and of episodes of otitis media respiratory tract infections as well as levels of serum immunoglobulins were followed during [redacted] months after adenoidectomy in a consecutive group of 274 children The total incidence of atopic disease was high (23 6%) at the start of the study and increased further [redacted] 39 0% during the study Increased serum IgE levels positive RAST tests and/or positive provocation tests were found before the onset of atopic symptoms in [redacted] out of the 19 children developing such symptoms during the observation period Otitis media continued to occur in 42 9% of the children The incidence of episodes of otitis media after the adenoidectomy was higher with lower age a high number of episodes of otitis media before the operation and/or a history of atropic disease None of the laboratory tests could predict subsequent episodes of otitis media Protracted respiratory tract infections developed only in children with laboratory findings indicative of atopy Serum IgF and IgM levels decreased significantly No serious infections and no dysgammaglobulinaemias developed Adenoidectomy seems to be a rather minor trauma from an immunological point of view but further and controlled studies are needed concerning the possible clinical benefit of adenoidectomy in children with recurrent otitis media

KEY WORDS Children allergy otitis media adenoidectomy IgE IgM RAST

Adenoidectomy is performed in about 25% of Swedish children (28) It is supposed to eliminate nasal obstructive symptoms as well as preventing recurrencies of otitis media (6) However a recent investigation (26) failed to show significant differences in the incidence of episodes of otitis media between children submitted to adenoidectomy and a non operated control group with the same initial symptoms Consequently a more conservative attitude towards adenoidectomy was recommended Furthermore it has been shown that local defence mechanisms may deteriorate after removal of lymphoid tissue (22) Children with frequent episodes of otitis media often have symptoms of allergic disease (14 16 27) Such children should benefit from allergological treatment rather than from adenoidectomy (30) It has even been suggested that adenoidectomy might intensify or even initiate allergic symptoms

The purpose of the present study was to find out if certain symptoms or laboratory findings can predict the effect of adenoidectomy if severe infections and/or immunoglobulin deficiencies develop after such an operation if allergic symptoms develop or deteriorate and if so if laboratory tests can predict the development of such allergic symptoms

MATERIAL AND METHODS

A consecutive group of 274 children 1–16 (mean=5 8) years of age was followed for 16 months after adenoidec

Table 1 *Results of the initial investigation*

	Investigated	Positive n	Positive %
Family history of recurrent otitis media	244	100	41 0
Family history of atopic disease	254	120	49 2
Atopic disease	259	61	23 6
IgE>+2 S D for age	150	22	14 7
RAST test class 2–4	150	12	8 0
Pos skin and provocation test	134	33	24 6
Blood eosinophilia	132	33	25 0
Two or more laboratory findings indicative of atopy	149	59	39 6
Bacterial swabs	135	62	45 9

tomy Case histories were obtained from records at the Ear Nose and Throat and Pediatric Departments and from questionnaires at the start ($n=259$) and follow up ($n=225$) In these the main emphasis was placed on the family history (family=parents and siblings) and on adenoid symptoms as well as on the susceptibility to infections and occurrence of atopic disease (=bronchial asthma allergic rhinitis atopic dermatitis allergic urticaria) Furthermore the smoking habits and presence of pets in the family were determined Medical records were scrutinized for episodes of otitis media during the follow up period in all 274 children

All 94 children with recurrent otitis media (=otitis media 4 times or more before the adenoidectomy) and 60 children with mainly nasal symptoms selected at random were submitted to an allergological and immunological study (13) This included skin and conjunctival tests (1) using 17 allergens serum IgE (Phadebas® IgE test) specific IgE antibodies (Phadebas RAST®) against 12 allergens and blood eosinophils (9) Serum immunoglobulins A and G were analysed by electroimmunoassay (15) and IgM by single radial immunodiffusion (17) Nasopharyngeal swabs were taken at the adenoidectomy and were analysed for the occurrence of *Diplococcus pneumoniae* and *Haemophilus influenzae* A second blood sample was drawn from 123 children at the end of the follow up period

Lab atopy (=laboratory findings indicating atopy) was defined as two or more of the following findings serum IgE level more than two standard deviations (S D) above the mean for that age (10) positive skin and conjunctival tests positive RAST test and blood eosinophilia (>5%)

Immune deficiency any of IgA IgG or IgM less than two S D below the mean for that age (3)

Group differences were evaluated using the χ^2 test and Student s t test

RESULTS

The adenoidectomies were performed without serious complications one child was reoperated upon on the second day because of prolonged bleeding

There was a high incidence of atopic disease (23 6%) and of lab atopy (39 6%) in the initial material (Table I) A positive bacterial culture was obtained from the adenoid in 45 9% of the children without significant differences between findings in atopic and non atopic children

Otitis media snoring protracted upper respiratory tract infections (=URI s) and frequent URI s were less common after than before the adenoidectomy (Fig 1) A minor no of children developed such symptoms without having had them before

The calculated association (χ test) between background factors such as the family and case histories and laboratory findings and the occurrence of symptoms after the adenoidectomy is presented in Table 2

Otitis media occurred in 80 (29 2%) of the 274 children after the adenoidectomy Eight of these 80 had otitis media as often as 4 times or more The children who continued to have episodes of otitis media were younger (3 9±2 4 years of age) than those in whom such episodes came to an end (5 6±3 4 years $p<0.001$) There was a statistically significant correlation ($p<0.001$) between the number of episodes of otitis media before and after the adenoidectomy (Fig 2)

A family history of recurrent otitis media oc

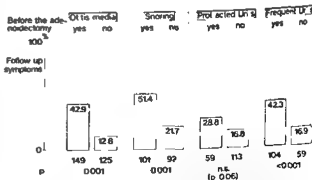


Fig 1 Symptoms during the follow up period in relation to those before the adenoidectomy *Yes* indicates that all children had the symptom before the adenoidectomy *No* indicates that the symptoms had not occurred before the adenoidec

Table 2 *Symptoms after the adenoidectomy in relation to initial findings*
Cross tabulation of calculated associations (see text)

	Otitis media	Snoring	Protracted Uri s	Frequent Uri s
Age	<0 001	n s	n s	n s
Family history of recurrent otitis media	<0 05	n s	<0 05	n s
No of episodes of otitis media	<0 001	n s	n s	n s
Lab atopy	n s	n s	<0 01	n s
Atopic disease	<0 05	n s	n s	n s

curred more often among children who continued to have otitis media than in those who did not ($p<0.05$) Atopic disease was over represented in children who had their first episode of otitis media during the follow up period ($p<0.05$)

Protracted Uri s developed more often in children with a family history of recurrent otitis media than in children without such a family history ($p<0.05$) All 13 children who developed this symptom after the adenoidectomy had laboratory findings indicative of atopy ($p<0.01$) No child developed serious infections after the adenoidectomy No significant connection was found between otitis media snoring protracted or frequent Uri s during the follow up period and the following background findings sex family history of atopic disease smoking habits of parents presence of pets at home a positive bacterial culture or a finding of immune deficiency at the adenoidectomy

Serum immunoglobulins Numerically serum IgG levels increased and IgM levels decreased significantly ($p<0.001$) between the two samplings whilst no significant change occurred in the IgA levels However most children with initial levels of IgG and/or IgM more than two S D above the mean for that age showed IgG and IgM levels between +2 and −2 S D from the mean at the follow up investigation (Table 3) but there was no tendency to the development of dysgammaglobulinemias No predictive information was obtained from the initial serum levels of IgG or IgM No difference was found in the follow up levels of these immunoglobulins between children with and those without otitis media after the adenoidectomy Four children with initial serum IgA levels less than two S D below the mean for that age had otitis media after the adenoidectomy whilst no such episodes occurred in six other children with low initial IgM or IgG levels

Serum IgE levels (Fig 3) showed a significant decrease ($p<0.001$) between the first (91 1±119 kU/l) and the second (69 6±81 4 kU/l) sampling There was no significant difference in the IgE level between children with or without otitis media after the adeoidectomy (Table 4) The initial RAST test was positive in 12 children nine of these showed symptoms of atopic disease on the initial investigation and the remaining three developed such symptoms during the follow up period

Skin and provocation tests Children with positive initial tests in the absence of symp

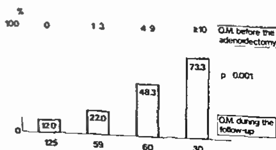


Fig 2 Incidence of otitis media (O M) during the follow up period in relation to the number of such episodes (0–≥10) before the adenoidectomy

Table 3. *Serum immunoglobulin levels at the start and follow up investigations*

	Start levels							
	IgG				IgA			
Follow up levels	>+2 S D	<+2 S D >−2 S D	<−2 S D	Total	>+2 S D	<+2 S D >−2 S D	<−2 S D	Total
>+2 S D	2	8	0	10	1	9	0	10
<+2 S D >−2 S D	22	90	0	112	4	104	1	109
<−2 S D	0	0	1	1	0	0	4	4
Total	24	98	1	123	5	113	5	123

toms of atopic disease ($n=16$) developed such symptoms (5/16) more often ($p<0.05$) during the observation period than those with negative tests (7/70). The result of these tests gave no predictive information as to subsequent episodes of otitis media.

Atopic disease developed in 19 (15.4%) of the 123 children whose blood was sampled twice. Positive laboratory findings (Table 5) preceded the onset of atopic symptoms in 13 (68.4%) of these children. The new atopic symptoms were moderate or mild. No child developed severe atopic symptoms during the observation period. No previous atopic disease deteriorated but six children with atopic dermatitis acquired moderate allergic rhinitis during the follow up period. At the end of the study 48 children (39.0%) had a diagnosis of atopic diseases.

DISCUSSION

The incidence of snoring, frequent URIs, protracted URIs and otitis media was lower after the adenoidectomy than before this operation. In the absence of a non operated control group no conclusions can be drawn from this improvement. Most cases of otitis media are preceded by a viral URI. The incidence of URIs decreases with age (21) and a corresponding decrease in the incidence of otitis media should thus be expected. In fact the incidence of otitis media decreased with age from a peak at 3–6 years of age in an extensive UK survey (20).

A majority of the children with otitis media prior to the adenoidectomy remained free from otitis media during the observation period. However, otitis media continued to occur in 42.9% of the children in our study as compared to 40% in a similar study (4). Predisposing factors were many episodes of otitis media before the operation and a family history of such disease.

Atopic disease seemed to increase slightly the risk of developing otitis media (Table 2). Atopy implies a readiness to produce IgE antibodies on ordinary environmental exposure to common allergens (23), but also a tissue hyperreactivity (25) which in some cases may

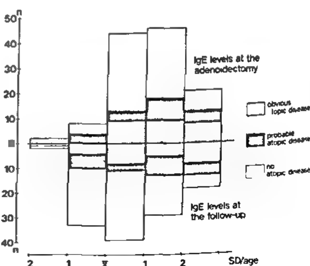


Fig. 3. Serum IgE levels at the initial and follow up investigations.

...+2 S D	<+ S D >- S D	<-2 S D	Total
	1	[illegible]	3
	95	2	114
	5	1	6
	101	3	123

be the most important drawback This hyper reactivity implies an increased inflammatory swelling during viral infections thus clearing the way for bacteria The increased incidence of otitis media in atopic subjects has been emphasized by others (2 8)

The incidence of otitis media was nearly identical in adenoidectomized and non adenoidectomized subjects in an open study (20) There are few controlled studies on the effect of an isolated adenoidectomy However Rynell Dagoö & Shirazki (26) found no decrease in the incidence of otitis media and Un s attributable to adenodectomy A combined adenotonsillectomy did not decrease the incidence of otitis media in another study (18 19)

The incidence of immunoglobulin deficiencies was low (10/150) in our study as compared to findings by others (6 7) There was no development of dysgammaglobulinaemias No serious infections were found during the follow up period nor have such infections been reported by others in spite of certain post operative laboratory findings (22)

The serum IgE levels decreased significantly during the study This might be due to the removal of a great number of IgE producing plasma cells present in adenoid tissue (29) However it is more probable that this decrease is the result of a decreased stimulation of the immune system by removing a chronically infected adenoid possibly also by

Table 4 *Change in serum IgE levels in relation to the occurrence of atopic disease and otitis media during the follow up period*

Change=increase or decrease by 1 S D or more in relation to age

Change in IgE level	Atopic disease			No atopic disease			Total	
	n	Otitis media	No otitis	*n*	Otitis media	No otitis	*n*	%
Increase	9	[illegible]	3	10	2	8	19	15 7
No change	23	1	11	5	17	8	48	39 7
Decrease	16	7	9	38	14	24	54	44 6
Total	48	5	23	73	33	40	1 1	100

Table 5 *Atopic disease in 123 children whose blood was sampled twice*

	Before the adenoidectomy	Acquired after the adenoidectomy		Total	
		n	Lab atopy	*n*	%
...onchial asthma	7	4	3	11	8 9
...llerg c rhinitis	9	19	11	8	2 8
...topic dermatitis	10	0	0	10	8 1
...llergic urticaria	10	3	2	13	10 6
...ne disease or more	9 (3 6)	19 (15 4)	11	48	39 0

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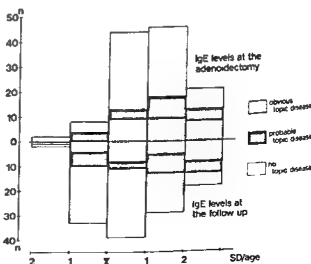


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fewer and shorter URIs Rather little is known about serum IgE levels in chronic or recurrent infections but it is plausible that serum IgE elevations in children with frequent respiratory infections (24) are the result of repeated antigenic stimulation exerted by the respiratory infections on the local IgE producing plasma cells The possible role of suppressor T cells on the serum IgE level and their behaviour in recurrent respiratory infections also remains to be clarified

There was a high incidence of atopic disease at the time of the adenoidectomy 29 (23 6%) of the 123 children whose blood was sampled twice had such disease This incidence is higher ($p<0.001$) than the 15 1% found in a group of unselected 7 year old school children in the same region (12) Nineteen (15 4%) new cases of atopic disease developed during the follow up period This incidence seems high but is not astonishing with regard to the high incidence of laboratory findings indicating atopy (39 6%) at the initial investigation A majority (13/19) of these new cases had laboratory findings indicative of atopy before specific symptoms developed This is in accordance with our findings in other groups (11) and also agrees with the findings of others (5)

The possibly provocative role of the adenoidectomy cannot be supported in the absence of a non operated control group

In conclusion adenoidectomy did not seem to stop recurrencies of otitis media in children with a history of many such episodes Otitis media continued to occur especially in the youngest children A history of atopic disease also increased the incidence of otitis media On the other hand there were no serious infections or new cases of dysgammaglobulinaemias after the adenoidectomy

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BACTERIAL MENINGITIS IN CHILDHOOD IN AN AFRICAN CITY

Factors Influencing Aetiology and Outcome

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ABSTRACT Hailemeskel H and Tafari N (Ethio-Swedish Paediatric Clinic and the Department of Paediatrics Addis Ababa University Addis Ababa Ethiopia) Bacterial meningitis in childhood in an African city Factors influencing aetiology and outcome Acta Paediatr Scand 67 725 1978 —In a retrospective study of 120 children aged 1 month and above with bacterial meningitis confirmed by positive CSF culture 88 4% were found to be due to three common organisms *H influenzae Str pneumoniae* and *N meningitidis* Gram-negative enteric organisms accounted for 10% of the infections Despite intensive antibiotic and ancillary therapy there has been no significant change in case fatality and sequelae over the past decade in this institution The present study confirms that factors related to the organism and the host are important in determining the outcome of therapy *H influenzae* and *Str pneumoniae* infections are associated with statistically significant rise in case fatality rate and neurologic sequelae at the end of therapy The presence of neurological abnormality at the time of diagnosis significantly increases case fatality rate while delay in diagnosis appears to primarily influence the frequency of neurological sequelae Protein-energy malnutrition [redacted] creases the frequency of neurological sequelae and death from bacterial meningitis without significantly influencing the pattern of bacterial aetiology The finding of enteric Gram negative meningitis in association with diarrhoeal disease in the present study adds a new dimension to one of the most prevalent health problems in developing countries and needs to be confirmed

KEY WORDS Bacterial meningitis diarrhoea protein-energy malnutrition developing countries

Reports from developing countries indicate excessive case fatality rates in bacterial meningitis (2 4 5 9 23) Although reliable data on the incidence of bacterial meningitis in developing countries are not available the risk of contracting the infection has been shown to increase when factors associated with poverty are present (7 14) The purpose of the present report [redacted] to examine factors related to aetiology and the outcome of therapy in bacterial meningitis in infancy and childhood in an African city where high prevalence of infection and protein energy malnutrition are encountered

PATIENTS AND METHODS

The patients were consecutive admissions with bacterial meningitis to the Ethio-Swedish Pediatric Clinic[1] between January 1 1975 and December 31 1976 The criterion for inclusion in the study was bacterial growth on cerebro spinal fluid (CSF) culture Infants aged less than one month were not included as these were planned to be the subject of separate report

The records of 120 infants and children aged between one month and 14 years were examined for bacterial aetiology duration of illness prior to diagnosis the presence of recognizable neurologic symptoms specifically alterations in the level of consciousness seizures signs of supratentorial mass lesion (24) nutritional status (20) and other coincidental disorders

All patients were treated uniformly along guidelines established previously (18) Treatment was initiated with crystalline penicillin [redacted] and chloramphenicol until culture results were known The route and dosage of these were as follows crystalline penicillin G 500 000 u/kg/day intravenously in 8 divided doses chloramphenicol 100 mg/kg/

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[1] The Ethio-Swedish Paediatric Clinic is the major referral centre for infants and children under the age of 14 years living in the city of Addis Ababa and its immediate environs

Table 3 *Factors associated with outcome of therapy in bacterial meningitis*

	N	Outcome (%)			P
		Intact survival	Sequelae	Death	
Age					
≤12 months	95	52 2	20 0	27 8	<0 05
>12 months	25	63 0	29 6	7 4	
Organism					
H influenzae	50	45 8	35 4	18 8	
Str pneumoniae	26	46 2	15 4	38 5	<0 01
N meningitidis	30	76 7	16 7	6 7	
Neurological abnormality at diagnosis					
Present	60	31 0	31 0	38 0	<0 001
Absent	60	76 3	16 9	6 8	
Duration of illness					
≤72 hours	43	69 0	9 5	21 4	<0 025
>72 hours	77	44 7	31 6	23 7	
Weight for age					
≥80% standard	82	62 2	19 5	18 3	<0 0 5
<80% standard	38	43 3	34 3	31 4	
Subdural fluid					
Present	32	31 3	31 3	37 5	<0 01
Absent	88	65 9	17 0	17 0	
All patients	1 0	51 2	6 8	22 0	–

countered in 42 patients the diagnoses included 11 different disorders (Table 2) Twelve of the 13 cases of diarrhoea were in infants with Gram negative bacterial meningitis The initial diagnosis in all 12 patients was dehydration following acute gastroenteritis and the diagnosis of meningitis was established subsequently All patients apparently contracted the infection outside the hospital environment except 5 in whom meningitis was diagnosed after the first day of hospitalization Two of these were hospitalized elsewhere and after transfer one was found to have Salmonella Group [redacted] and the other *A calcoaceticus* var *anitratus* meningitis The remaining 3 patients were diagnosed to have bacterial meningitis sometime after the third day of hospitalization and in these *Enterobacter cloacae* was isolated in 2 and *A calcoaceticus* var *anitratus* in 1 In all cases of Enterobacteriaceae and Acinetobacter meningitis the same organism was also isolated from the blood

Subdural effusion and empyema Subdural fluid collections were encountered in 32 (26 6%) patients The oldest patient was 19 months old No case of subdural fluid collection was encountered in *N meningitidis* meningitis while the frequency of *Str pneumoniae* and other Gram positive meningitis was increased 1 6 fold and 1 9 fold respectively when subdural fluid collection complicated meningitis ($p<0\ 001$) Subdural fluid in 16 patients yielded the same organism initially isolated from the CSF

Outcome of therapy The case fatality rate in the present series was 22% Thirty two (26 6%) patients had recognizable neurologic abnormality at the end of therapy and recovery without apparent sequelae was noted in 61 (51 2%) patients (Table 3) The frequency of neurological sequelae and death from bacterial meningitis showed statistically significant association with age aetiologic agent duration of illness prior to diagnosis presence of neurological abnormality subdural fluid collection and protein-energy malnutrition

Case fatality rate was 3 [redacted] times higher in infants under the age of one year than in those

Table 1 *Aetiology of bacterial meningitis in Addis Ababa (1975–1976)*

Bacteria	No of cases	%
H influenzae	50	41.7
N meningitidis	30	25.0
Str pneumoniae	26	21.7
Enterobacteriaceae		
Salmonella Group B	4	3.3
E cloacae	3	2.5
Klebsiella spp	1	0.8
Esch coli	1	0.8
Acinetobacter		
A calcoaceticus var anitratus	2	1.7
A calcoaceticus var lwoffi	1	0.8
Streptococcus (ungrouped)	2	1.7
Total	120	100.0

day intravenously or orally in 4 divided doses The route and dosage of the above antibiotics were maintained throughout the duration of therapy which was for a minimum of 10 days regardless of the rapidity with which the patient responded and continued beyond 10 days if symptoms persisted or the CSF failed to become normal Antibiotics for the treatment of bacterial meningitis due to unusual organisms were selected according to sensitivity results Kanamycin and gentamycin were the antimicrobials frequently used Ancillary treatments included prevention of the syndrome of inappropriate ADH secretion by fluid restriction correction of symptomatic hyponatraemia with 3% saline osmotic decompression of acute brain swelling with 25% mannitol 1 g/kg/dose and monitoring for the development of subdural effusion and routine subdural tapping in suspected cases All subdural collections were subsequently cultured and the fluids classified into sterile effusions infected effusions and empyema For lack of satisfactory definition infected subdural effusions and empyemas were combined in this presentation

Standard methods were used in primary isolation and identification of bacteria (8) The chi square test for trend in proportions (1) was used in the analysis of data

RESULTS

Bacterial aetiology The three organisms commonly associated with childhood bacterial meningitis accounted for 88.4% of the infections (Table 1) Fourteen (11.6%) had meningitis due to other organisms Age sex and the presence of complicating illness other than protein energy malnutrition seem to be significantly associated with bacterial aetiology of meningitis (Table 2) The frequency of *H influenzae* decreased 7 fold while the frequency of *N meningitidis* increased 9.6 fold after the age of one year All cases of meningitis due to unusual organisms occurred in infants aged under one year The male to female ratio in the present series of 1.4 : 1.0 is the same as the sex ratio of admissions to this hospital However males exceeded females 5 fold in meningitis due to *Str pneumoniae* while females exceeded males 1.7 and 1.4 times in *N meningitidis* and *H influenzae* meningitis respectively Complicating illnesses were en

Table 2 *Factors influencing bacterial aetiology of pyogenic meningitis in infants and children*

	N	Bacterial aetiology (%) *H influenzae*	*Str pneumoniae*	*N meningitidis*	Other	*p*
Sex						<0.01
Male	71	37.0	31.5	19.2	12.3	
Female	49	50.0	6.3	33.3	10.3	
Age						<0.001
≤12 months	95	52.1	24.5	8.5	14.9	
>12 months	25	7.4	11.1	81.5	–	
Complicating disease						<0.05
Present	42	42.9	14.3	19.0	23.8	
Absent	78	41.8	25.3	27.8	5.1	
Subdural effusion/empyema						<0.001
Present	32	43.8	34.4	–	21.9	
Absent	88	40.9	17.0	34.1	8.0	
All patients	120	42.1	21.5	24.8	11.6	–

Excluding protein-energy malnutrition These were anaemia 14 diarrhoea 13 pneumonia 9 hepatitis 4 rickets 3 one of each of measles laryngotracheobronchitis scabies giardiasis ascariasis and strongyloidiasis

was no significant relationship between duration of symptoms and the level of bacterial concentration in the CSF This may explain the observation in the present study that case fatality rate did not increase appreciably when the duration of illness prior to diagnosis was longer than 72 hours

Sterile subdural effusion was found in 13 3% of the cases and as these constituted subdural taps on symptomatic cases and transillumination was not routinely done the true incidence of the complication in the present study is unknown Cases classified as subdural empyema (13 3%) may represent true empyemas or infected subdural fluids Infection of a subdural effusion often leads to empyema in infants but in older children and adults subdural empyema usually occurs after otorhinologic infections (6 10) The finding of increased case fatality rate and sequelae in the present series in cases of subdural effusion complicating acute bacterial meningitis is consistent with previous observation (3)

Bacterial meningitis in infancy and childhood in developing countries continues to be a serious health problem attended by excessive case fatality and neurologic sequelae rates (2 4 5 9 23) Energy protein malnutrition significantly increases morbidity and mortality from bacterial meningitis in childhood although the mechanisms for this are not clear The high frequency of bacterial diarrhoea in developing countries may further contribute to the relatively high frequency of Gram negative bacterial meningitis of enteric origin in infancy beyond the newborn period The effectiveness of early diagnosis and prompt appropriate antimicrobial therapy are obviously limited (11 12 17) and the development of vaccines is the only hopeful measure in controlling this devastating illness under conditions prevailing in most third world countries

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aged one year and above The highest case fatality rate was seen in *Str pneumoniae* (38 5%) and the lowest in *N meningitidis* (6 7%) meningitis The 5 6 fold increase in case fatality rate observed in patients with neurological abnormality at the time of diagnosis showed significant association ($p<0\ 05$) when the duration of illness was held constant Duration of illness on the other hand showed statistically significant association with neurologic sequelae in that the frequency of such abnormalities was increased 3 3 fold in patients in whom the duration of illness was prolonged beyond 72 hours The frequency of death and neurological sequelae were increased 1 7 fold and 1 8 fold when the patients manifested clinically significant protein energy malnutrition ($p<0\ 025$) Subdural effusions and empyema were significantly associated with increased frequency of neurological sequelae and death from bacterial meningitis ($p<0\ 01$)

DISCUSSION

The bacterial aetiology of meningitis in the present study is in agreement with most recently published series (7 11 15 17) As noted elsewhere (15 17 21 27) *H influenzae* has replaced *Str pneumoniae* (9) as the most frequent organism isolated from infants and children with meningitis Meningitis due to Gram negative enteric organisms in 12 of the 120 patients (10%) in the present series is one of the highest reported in recent years Salmonella meningitis in infancy and childhood has been noted in epidemics (25) while *Enterobacter cloacae* Klebsiella and Acinetobacter species have been implicated in nosocomial infections (15) Although infection with unusual organisms in malnourished children have been documented (22) only 3 of the 12 patients with enteric Gram negative bacterial meningitis in the present series were malnourished one infant had kwashiorkor and 2 were between 70 and 80% of the standard weight for age (20) The universal occurrence of diarrhoea and septicaemia in these patients suggest that the portal of entry of these organisms might have been the gastrointestinal tract and that meningitis was secondary to localization after a haematogenous spread Enteric Gram negatives other than *Esch coli* have recently been shown to cause diarrhoea in infants and children through the production of enterotoxin (19 28) Some of these bacteria may also have invasive properties (26)

There is no appreciable change in case fatality rate in bacterial meningitis since the last report from this hospital a decade ago (9) phenomenon also noted by recent reports from North America and Europe (11 12 17) Factors related to the infecting organism and the host are undoubtedly important in determining the outcome of the therapy Case fatality rate and frequency of neurological sequelae in the present series was higher in *Str pneumoniae* and *H influenzae* while recovery without apparent sequelae were most often observed in *N meningitidis* meningitis The influence of age on the outcome of therapy can be explained on the frequency of these organisms 76 6% of meningitis in the first year were due to *H influenzae* and *Str pneumoniae* while 81 5% of meningitis beyond the first year were due to *N meningitidis* Statistically significant influence on case fatality and the presence of neurological sequelae at the end of therapy was noted when there was neurological abnormality at the time of diagnosis when diagnosis was delayed beyond 72 hours after the onset of symptoms and when there was evidence of protein energy malnutrition (Table 3) Some of the features observed in the present study find their explanation in a recent study by Feldman (13) who showed significant correlation between CSF bacterial concentration at the start of therapy and outcome In that study bacterial concentration in CSF were significantly higher in *H influenzae* and *Str pneumoniae* infections than in *N meningitidis* infections Seizures and subdural effusions were seen significantly more frequently in those patients whose CSF bacterial count was greater than 10^7 per ml However there

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EMPYEMA IN CHILDREN IN TROPICS

ASHFAQ A KHAN B M GUPTA J OLONGA and M J MAGUIRE

From the Children s (University Teaching) Hospital Lusaka Zambia

ABSTRACT Khan Ashfaq A Gupta B M Olonga J and Maguire M J (Childrens University Teaching Hospital Lusaka Zambia) Empyema in children in tropics Acta Paediatr Scand 67 731 1978 —Thirty six cases of empyema were diagnosed in children over a two year period giving an overall incidence of 0 11 % of the total hospital admissions Many of them had measles bronchopneumonia and most were malnourished 16 children had penicillin resistant staphylococcal infection 29 of them were treated with closed tube drainage Thirteen died during the course of treatment the majority within three days of admission The rest were followed up over a period of up to six months and showed good recovery Six of these patients developed pneumothorax during the course of treatment Various combinations of Ampicillin Cloxacillin Gentamycin and Cotrimoxazole parenterally were used Supportive treatment with blood transfusion was found to be beneficial

KEY WORDS Empyema measles bronchopneumonia malnutrition *staphylococcus aureus* closed tube drainage

Empyema was a frequent complication of pneumonia in childhood before the advent of antibiotics The common infecting organisms were the pneumococcus and streptococcus The incidence of empyema dropped to almost negligible levels in the early period of antibiotic use so that the disease was considered to be extinct (11) However over the last two decades an increasing number of cases of empyema have been reported from developed countries The commonest organism reported now is the penicillinase producing staphylococcus (1 5)

Empyema in tropical countries is seen occasionally (12) but little information is available regarding its incidence bacteriology and morbidity At the Children s Hospital Lusaka only a few patients of empyema were diagnosed previously Recently there has been a steady rise in the frequency of this condition

MATERIALS AND METHODS

A study is presented of thirty six children suffering from empyema admitted over two years in 1976 and 1977 to the Children s (University Teaching) Hospital Lusaka Diagnosis was confirmed by aspiration of purulent fluid from the pleural cavity Pus was cultured for organisms and sensitivity Tubercular effusions were excluded from this series A detailed history physical and laboratory examinations and repeated X rays were done in each case A record was made of antibiotics given before and during therapy

RESULTS

A total of thirty six patients of empyema were diagnosed in children over a period of two years Their ages ranged from one month to five years (Table 1) There were 16 infants below one year 18 in the age group of 1–3 years and two cases over 3 years of age Majority of these children were diagnosed between the months of December and April in both years

Clinical manifestation Most patients had a history of an infection of 2–6 weeks duration (Table 2) Empyema was most frequently a complication of measles bronchopneumonia (16 cases) Three patients had pustular skin infection including one who had infected Tumbu fly (cordylobia anthropophaga) lesions over the scalp and hands Four had recurrent attacks of bronchopneumonia and had been admitted twice in hospital Thirteen pa

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Table 1 *Age and sex distribution of empyema patients*

Number of deaths is shown in brackets

Age groups	0–3 months	–1 year	–3 years	Over 3 years	Total
Male	4 (2)	4 (2)	11 (4)	2 (1)	21 (9)
Female	3 (2)	5 (1)	7 (1)	–	15 (4)
Total	7 (4)	9 (3)	18 (5)	2 (1)	36 (13)

Table 3 *Nutritional status*

Nutritional status	No of patients
Average weight for age	10 (27 8%)
Moderate malnutrition	13 (36 1%)
Severe malnutrition	13 (36 1%)

According to Harvard standard

tients had acute bronchopneumonia with symptoms of 3–7 days duration

Clinical signs of bronchopneumonia were present in most of the patients All of them showed marked prostration pallor and were toxic One infant had paralytic ileus and another had marked cellulitis of the left thigh Four patients had an associated malarial infection and one had also a urinary tract infection In 23 children the diagnosis of empyema was suspected on the initial clinical examination and the rest were diagnosed radiologically

The nutritional status of these children had an important bearing on the development of empyema Infants below three months of age were within normal limits of weight for age the rest (Table 3) were malnourished with weight for age less than 80% of Harvard standards Thirteen were suffering from severe malnutrition with signs of kwashiorkor

None of the children had a chest deformity finger clubbing a draining sinus or other evidence of chronic lung disease

Radiological and laboratory findings X ray of the chest was most useful in establishing the diagnosis (Table 4) There were 19 children with left sided empyema Ten of the patients had pyopneumothorax and one had pyopericardium Diagnostic aspiration was done on each child after radiological examination A variable amount of pus or opalescent fluid (Few ml to 150 ml) was aspirated with a wide bore needle size 19

Bacteriological data from 32 cases out of 36 patients are presented in Table 5 Four patients were referred for emergency surgery and culture results were not available From half of the patients staphylococcus aureus was cultured and of these 16 were found to be resistant to penicillin There was no growth in eleven cases and all of them had received some antibiotics prior to the hospital admission

Blood culture was done in twenty patients and of these three grew staphylococcus aureus which again was resistant to penicillin

16 children had a marked polymorphic leucocytosis and 18 had anaemia on admission with haemoglobin levels less than 80 g/l In all patients there was rapid fall in the haemoglobin during the acute stage of the disease

Treatment 29 out of 36 children had closed tube drainage with underwater seal The tube

Table 2 *Precipitating infections*

Infection	No of patients
Measles	16 (44 5%)
Pustular skin eruptions	3 (8 3%)
Unresolved pneumonia	4 (11 1%)
Acute bronchopneumonia	13 (36 1%)

Table 4 *Radiological findings*

	Clinical and X ray diagnosis	No
Left side	Empyema	13 (36 1%)
	Pyopneumothorax	8 (16 6%)
Right side	Empyema	12 (33 3%)
	Pyopneumothorax	4 (11 1%)
	Empyema with pyopericardium	1 (2 8%)

Table 5 *Bacteriology and sensitivity pattern*

3 patients

Organism	Sensitivity pattern	No of patients
Staphylococcus (17 patients)	Sensitive to penicillin group	2
	Partial sensitivity to penicillin group	2
	Resistant to penicillin group	13
Pneumococcus (1 patient)	Sensitive to penicillin	1
Mixed organisms	1	
a Streptococci	Sensitive to penicillin	1
b Staphylococci	Resistant to penicillin group	1
Coliform (1 patient)	Resistant to penicillin and tetracyclin	1
Streptococcus viridans	1	1
Sterile Culture	–	11

was kept in situ for a period of 2–6 days None of the children required open drainage rib resection or pleurectomy Three had scanty amount of fluid and tube drainage was not instituted

Antibiotics used in various combinations were ampicillin and cloxacillin (39%) ampi clox and gentamycin (11%) cephradine (11%) and co trimoxazole (28%) The dosage schedule was based on mg/kg adjusted to weight as recommended by manufacturers Duration of treatment was from 3–8 weeks Blood transfusion was given to all children within a week of admission since all of them became severely anaemic in hospital One child who developed a pyopericardium was successfully treated by open surgery and made a good recovery

In the children who recovered toxemia subsided a few days after admission following drainage of pus blood transfusion and antibiotics Low grade intermittent pyrexia interspersed with short spikes of high grade pyrexia was present for 2–3 weeks in 25 patients despite marked clinical improvement

Pneumothorax occurred in six cases during the course of treatment Three had to have repeat drainage after initial resolution Radiological clearance of pleura and lung expansion took 3–4 months One patient who developed pneumothorax refused further treatment but was reviewed after six months He showed complete radiological healing and resolution on subsequent examination

Mortality There were thirteen deaths in this series This high mortality was in the very small infants and in malnourished children with respiratory complications of measles Four children who were practically moribund died soon after admission Three had severe anaemia and died inspite of blood transfusion and supportive therapy One child had a large pneumocyst with cellulitis of the left thigh and he died most likely due to severe septicaemia

DISCUSSION

In this series of 36 empyema patients the majority of cases were found in children below three years of age as has been noted elsewhere (1 5 11 12) Incidence of empyema amongst paediatric admissions was 0 11% in both years The maximum number of admissions for measles and malnutrition is from December to April each year (8) when the children have low vitality and poor resistance 26 empyema patients were seen in these months 16 children had measles complicated by bronchopneumonia Bechamps et al (1) reported only 23% of their patients having history of preceeding infection Empyema complicated with measles has also been reported from India (12) with an overall incidence of 11 4%

In two large series of measles from East

Africa (4 9) there is no mention of secondary empyema Empyema was reported only in two cases in paediatric patients from a district hospital morbidity analysis in Uganda (2)

Empyema is on the increase in this hospital and organisms have shown resistance to the common antibiotics in routine use This confirms the experience of other reports (10) Inadequate therapy and delay in referral to hospital especially of children from low socio economic group increases difficulties of management and early effective therapy of respiratory infections

In developing countries staphylococcal infection is particularly common with measles bronchopneumonia and pustular skin infections (9 12) Staphylococcal pneumonia contributes to the high death rate and is associated invariably with resistance to the commonly used first line antibiotics (4) This is confirmed in our study as well Most patients (56%) had staphylococcal empyema Other organisms encountered in the present study were coliforms streptococci and pneumococci In eleven cases the empyema fluid was sterile but they too required closed tube drainage

Kevy & Love (7) are of the view that sterile fluid associated with thin pleural effusion most probably follow streptococcus infection Emerson et al (3) reported good recovery in cases with sterile cultures We do not share this view from our experience and this also was found in a Mayo clinic series (1) Sterile fluid is most likely due to prior inadequate treatment and the prognosis does not appear to be better in any way

In the preantibiotic era (5 6) rib section and thoracocentesis was a common procedure in the management of empyema This was due to the presence of thick pus found in pneumococcal empyema Closed tube drainage under water seal is now commonly practised (1 3 10) Adequate pleural drainage can be obtained without rib resection This is in contrast to adults and middle aged patients where Morin et al (10) showed success with early empyectomy

We found anaemia a common feature even after adequate treatment It was probably due to multiple factors including toxemia malnutrition and septicaemia Blood transfusion was given to all the patients as supportive therapy and to correct the anaemia

All patients in the present series who recovered had good lung expansion as was shown in serial X rays This indicates that there is better resolution and healing in children than in adults Decortication is rarely required and one should wait for a few months in follow up before a final decision is made to do so

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SELENIUM AND VITAMIN E IN CORD BLOOD FROM PRETERM AND FULL TERM INFANTS

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From the Paediatric Research Institute National Hospital of Norway Oslo and the Central Institute for Industrial Research Oslo Norway

ABSTRACT Hågå P and Lunde G (Paediatric Research Institute National Hospital of Norway Oslo and Central Institute for Industrial Research Oslo Norway) Selenium and vitamin E in cord blood from preterm and full term infants Acta Paediatr Scand 67 735 1978 —Selenium was determined in erythrocytes and serum and vitamin E and β lipoprotein in serum from cord blood samples of 31 full term and 20 preterm infants Venous samples from 21 mothers at birth and [illegible] normal adult women were also analyzed No difference for either selenium or vitamin E was found between the preterm and full term infants The selenium concentration in red blood cells was the same for newborn mothers at birth and normal adult women The serum concentration of selenium was however significantly lower in the newborn the mean level in the children being 64% of that in the mothers The level in the mothers did not differ from that in non pregnant women The vitamin E concentration was found to correlate very well with the β lipoprotein concentration This indicates that differences in the transport capacity account for the large difference in the serum tocopherol levels of mothers at birth and newborn

KEY WORDS Cord blood premature infants full term infants selenium vitamin E β lipoprotein

While a requirement for selenium is documented in animals no selenium deficiency symptoms have been described in humans A few children with kwashiorkor have however been reported to respond with increased weight gain on supplementation of the diet with selenium (13 21)

In 1973 selenium was shown to be an integral part of glutathione peroxidase (19) This enzyme plays a role in the protection of cellular membranes against oxidative degeneration as does vitamin E In rats the dietary intake of selenium affects the level of glutathione peroxidase in erythrocytes as well as in different tissues increased intake leading to higher enzyme activity (22) An interrelationship between tocopherol selenium and glutathione peroxidase has also been demonstrated Increased dietary intake of selenium significantly increases the plasma level of tocopherol both in rats and chicks (1 22) while vitamin E supplementation has been shown to increase the activity of glutathione peroxidase both in plasma erythrocytes liver and kidney of rats (22 25) In human newborn infants a positive correlation between the serum tocopherol and glutathione peroxidase activity of erythrocytes was noted by Emerson et al (2)

Vitamin E deficiency has been claimed as a cause of hemolysis in low birth weight premature infants during the first two months of life when the diets used have had a high content of polyunsaturated fatty acids and iron supplementation has been started very early (14 24)

The selenium status of the human individual may also affect the ability of the organism to prevent oxidative breakdown of the erythrocytes through its effect on the level of glutathione peroxidase as well as its effect on the plasma tocopherol level as has been observed in rats and chicks (1 22)

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KEY WORDS Cord blood premature infants full term infants selenium vitamin E β lipoprotein

While a requirement for selenium is documented in animals no selenium deficiency symptoms have been described in humans A few children with kwashiorkor have however been reported to respond *with increased* weight gain on supplementation of the diet with selenium (13 21)

In 1973 selenium was shown to be an integral part of glutathione peroxidase (19) This enzyme plays a role in the protection of cellular membranes against oxidative degeneration as does vitamin E In rats the dietary intake of selenium affects the level of glutathione peroxidase in erythrocytes as well as in different tissues increased intake leading to higher enzyme activity (22) An interrelationship between tocopherol selenium and glutathione peroxidase has also been demonstrated Increased dietary intake of selenium significantly increases the plasma level of tocopherol both in rats and chicks (1 22) while vitamin E supplementation has been shown to increase the activity of glutathione peroxidase both in plasma erythrocytes liver and kidney of rats (22 25) In human newborn infants a positive correlation between the serum tocopherol and glutathione peroxidase activity of erythrocytes was noted by Emerson et al (2)

Vitamin E deficiency has been claimed as a cause of hemolysis in low birth weight premature infants during the first two months of life when the diets used have had a high content of polyunsaturated fatty acids and iron supplementation has been started very early (14 24)

The selenium status of the human individual may also affect the ability of the organism to prevent oxidative breakdown of the erythrocytes through its effect on the level of glutathione peroxidase as well as its effect on the plasma tocopherol level as has been observed in rats and chicks (1 22)

Africa (4 9) there is no mention of secondary empyema Empyema was reported only in two cases in paediatric patients from a district hospital morbidity analysis in Uganda (2)

Empyema is on the increase in this hospital and organisms have shown resistance to the common antibiotics in routine use This confirms the experience of other reports (10) Inadequate therapy and delay in referral to hospital especially of children from low socio economic group increases difficulties of management and early effective therapy of respiratory infections

In developing countries staphylococcal infection is particularly common with measles bronchopneumonia and pustular skin infections (9 12) Staphylococcal pneumonia contributes to the high death rate and is associated invariably with resistance to the commonly used first line antibiotics (4) This is confirmed in our study as well Most patients (56%) had staphylococcal empyema Other organisms encountered in the present study were coliforms streptococci and pneumococci In eleven cases the empyema fluid was sterile but they too required closed tube drainage

Kevy & Love (7) are of the view that sterile fluid associated with thin pleural effusion most probably follow streptococcus infection Emerson et al (3) reported good recovery in cases with sterile cultures We do not share this view from our experience and this also was found in a Mayo clinic series (1) Sterile fluid is most likely due to prior inadequate treatment and the prognosis does not appear to be better in any way

In the preantibiotic era (5 6) rib section and thoracocentesis was a common procedure in the management of empyema This was due to the presence of thick pus found in pneumococcal empyema Closed tube drainage under water seal is now commonly practised (1 3 10) Adequate pleural drainage can be obtained without rib resection This is in contrast to adults and middle aged patients where Morin et al (10) showed success with early empyectomy

We found anaemia a common feature even after adequate treatment It was probably due to multiple factors including toxemia malnutrition and septicaemia Blood transfusion was given to all the patients as supportive therapy and to correct the anaemia

All patients in the present series who recovered had good lung expansion as was shown in serial X rays This indicates that there is better resolution and healing in children than in adults Decortication is rarely required and one should wait for a few months in follow up before a final decision is made to do so

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SELENIUM AND VITAMIN E IN CORD BLOOD FROM PRETERM AND FULL TERM INFANTS

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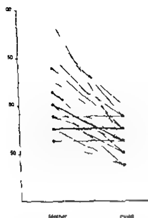


Fig 3 Selenium concentrations in sera of mothers at birth and newborn children The lines connect the individual mothers with their children

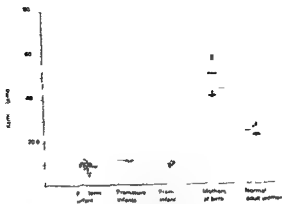


Fig 4 Vitamin E concentration in serum The horizontal lines are the means of the groups

significantly lower ($p<0.001$) than the serum concentrations in mothers at birth as well as the levels in normal adult women ($p<0.001$) On the other hand the serum concentrations in mothers at birth are not significantly different from the serum concentrations in normal adult women whose mean concentration was 1 39 μmol/l

The serum selenium levels in individual children are compared with the serum levels in their mothers in Fig 3 In 4 pairs the levels were equal while in all the others the child had a lower concentration than its mother mean level in the children being 64% of that in the mothers ($p<0.001$)

The serum vitamin E concentrations are shown in Fig 4 There is no statistical difference between premature and full term infants the mean serum tocopherol concentration in full term infants being 8 9 μmol/l The levels of tocopherol in normal adult women as well as in mothers at birth are significantly higher than in cord blood ($p<0.001$) while the mothers had significantly higher tocopherol concentration than the non pregnant women ($p<0.001$)

In 52 sera both the vitamin E and the β lipoprotein concentrations were measured In Fig 5 the relationship between them is shown The correlation is highly significant ($p<0.001$) Thus the low level of tocopherol in cord blood is associated with a low level of β lipoprotein while in the mothers tocopherol and β lipoprotein are concomitantly elevated

No significant correlations were found between vitamin E in serum and the selenium levels in either erythrocytes or in sera of newborn Nor was there any significant correlation between the levels found in the mother and those in her child for either selenium or vitamin E

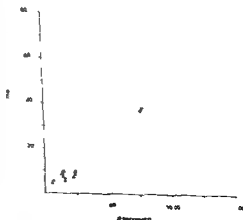


Fig 5 Vitamin E versus β lipoprotein concentration in serum ▲ newborn infants ■ adult women ● mothers at birth

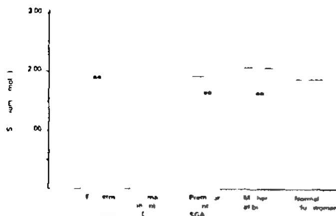


Fig 1 The selenium concentration in red blood cells The horizontal lines are the means of the groups

As part of a study of the early anemia of prematurity it was found of value to measure serum tocopherol serum selenium and red cell selenium concentrations in cord blood samples from premature infants and to compare them with the values obtained from cord blood of normal full term infants To see whether the values obtained from the child were affected by the levels of the mother blood samples were also obtained from some of the mothers shortly after delivery Adult non pregnant menstruating women were used as normal controls For a better assessment of the tocopherol values β lipoprotein was also measured

MATERIALS AND METHODS

Cord blood samples were obtained from 31 normal full term infants and 20 premature babies with birth weight less than 2000 g The premature infants were scored as appropriate for gestational age (AGA) or small for gestational age (SGA) according to the charts prepared by Gairdner et al (4) Venous samples from 21 mothers were drawn shortly after delivery and after consent was obtained The normal adult women used as controls were members of the hospital staff All samples were collected and stored in Vacutainer® tubes (Becton Dickinson) After separation the sera and red blood cells were kept frozen at −30 C until analysis

The *selenium* determinations were performed by neutron activation as previously described (12) The samples (about 2 ml) and selenium standards were irradiated with a neutron flux of 5×10^{12} *n/cm² sec for* 24 hrs Following irradiation the samples were transferred to inactive glass vials and the induced selenium activity ^{75}Se with a half life of 120 days was measured directly after allowing short lived radioactive nuclides to disintegrate A GeLi (33 cm³) detector was used for the registration

The vitamin E and β lipoprotein determinations were as a rule performed less than two weeks after sampling Free tocopherol in serum was determined by the fluorometric method of Hansen & Warwick (7) with minor modifications Duplicate serum samples of 100 µl were measured on a Perkin Elmer MPF III spectrofluorometer with the excitation wavelength set at 295 nm and the emission wave length at 324 nm β lipoprotein concentrations were measured by single radial immunodiffusion (M Partigen Behring Institut) The non parametric test of Wilcoxon was used for the statistical comparisons of the groups while Kendall s test was used to test correlations

RESULTS

The selenium concentrations in red blood cells (RBC) are shown in Fig 1 There is no difference in RBC selenium levels in premature and full term infants mean level for the full term infants being 1 89 µmol/l Moreover the content of selenium in the RBC of cord blood is the same as in the erythrocytes of mothers at birth as well as in those in normal adult women the mean level for the normal adult women being 1 89 µmol/l When the values of individual children were compared with those of their mothers no consistent pattern was detectable

The selenium concentrations of serum are depicted in Fig 2 The levels are the same in premature and full term infants the full term infants having a mean level of 0 66 µmol/l The serum levels in cord blood are however

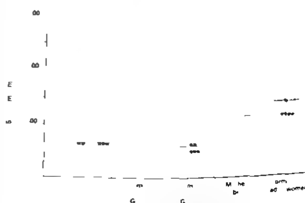


Fig 2 The selenium concentration in serum The horizontal lines are the means of the groups

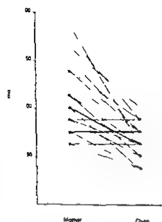


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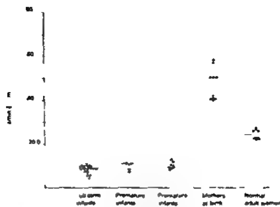


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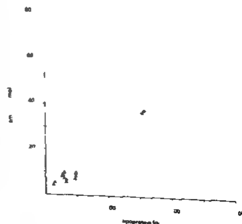


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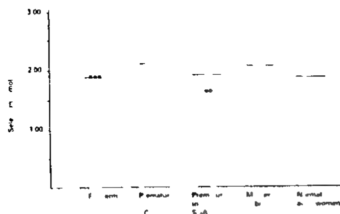


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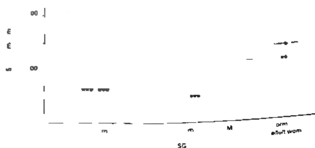


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DISCUSSION

Only few studies on the selenium status of newborn and pregnant women have been published (6 11 17) The serum selenium concentrations we have found in newborn mothers at birth and adult women agree well with findings in Germany and San Diego California (11 17) Erythrocyte glutathione peroxidase activity in newborn has been found to be low compared to adult levels in some studies (2 5) while others have failed to find any differences (11 18) The selenium bound to glutathione peroxidase probably accounts for about 60–75% of the total intra erythrocytic selenium (15 19) and in our study the red blood cell concentration of selenium was the same in newborn as in adults (Fig 1)

We found the selenium concentrations in serum and red blood cells from cord blood to be the same in premature and full term infants (Figs 1 and 2) The glutathione peroxidase activity of erythrocytes is also the same in preterm and full term infants (2 18) This does not appear to be compatible with the hypothesis that selenium and glutathione peroxidase deficiency is implicated in the early anemia of prematurity However the increased growth rate of the preterm infant may later give rise to a deficiency state particularly in artificially fed infants because the concentration of selenium in cow s milk is lower than in human milk (11) A fall in serum selenium and erythrocyte glutathione peroxidase has been observed both in full term infants (11) and in preterm infants (6) during the first months of life

Whether the drop occurs earlier and is more pronounced in premature infants and whether a fall of this magnitude will cause accelerated destruction of the red blood cells are the subjects of further investigations

While the red blood cells in the newborn contain as much selenium as those in the mother the serum selenium concentration is significantly lower than that in the mother (Figs 1 2 and 3) A possible explanation is that the supply of the element through the placenta is limited and that under such conditions the erythrocytes have a high priority Observations on the glutathione peroxidase levels of erythrocytes and liver in rats under dietary depletion of selenium support the latter hypothesis (16) While the glutathione peroxidase in liver fell to non detectable levels in 4 weeks the levels of the enzyme in red blood cells fell only slightly during a 10-week period

It is furthermore interesting to note that in protein energy malnutrition the same relationship between low serum and normal erythrocyte selenium values as in the newborn is observed (3 9)

The serum tocopherol results of this study showing low values in cord blood and raised levels in maternal serum are in accordance with other studies (10 23) The large difference between the levels of the mother and the child has customarily been attributed to a relative impermeability of the placenta for tocopherol

The vitamin E concentration in serum correlates with the level of circulating lipids and concurrent knowledge of the lipid level makes the serum vitamin E concentration a much more reliable index of the vitamin E status of the individual (8)

β lipoprotein is the principal carrier of tocopherol in plasma and the vitamin E level has been shown to correlate well with the β lipoprotein concentration (20)

This also holds true in cord blood and in the blood of mothers shortly after birth (Fig 5) Our data indicate that differences in transport capacity rather than a relative impermeability of the placenta for tocopherol is the reason for the low levels in newborn infants and raised maternal levels of tocopherol in serum

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EVALUATION OF THREE SPIROMETERS ON HEALTHY CHILDREN

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ABSTRACT Dalen G and Kjellman B (Departments of Paediatrics and Clinical Physiology Karnsjukhuset Skovde Sweden) Evaluation of three spirometers on healthy children Acta Paediatr Scand 67 741 1978 —Two electronic spirometers (Drager Spirotron and Monaghan M403) and one wedge bellows spirometer (Vitalograph) were compared with a Bernstein spirometer Healthy children 30 girls and 31 boys were investigated The regression lines of VC and FEV in relation to the body height to the third power are very close and the S D values around the lines are very similar The correlation coefficients of the regression lines are high for all the spirometers An analysis of the paired differences showed slight differences of the mean values The S D of paired differences was for VC 4 6–6 6% and for FEV 4 8–6 2% The PEFR values obtained by the two electronic spirometers deviated substantially and highly significantly from the values obtained by the Wright peak flow meter

KEY WORDS Healthy children spirometry electronic spirometers wedge bellows spirometer

Pulmonary function tests can supply information of additional value in connection with story and physical examination It would be advantageous if the paediatrician could use such tests in his office while examining children with pulmonary diseases Equipment to be used in this case must be simple and reliable and the procedure should not be time consuming The first step is often the use of simple flow meters e g Wright peak flow meter (6) The next step is usually spirometry with water spirometers e g the Bernstein spirometer The water spirometers are however too uncomfortable for most paediatricians The wedge bellows spirometers e g the Vitalograph and the new electronic spirometers have the advantages of simple operation and fast delivery of data Previous data from studies of adults seem to justify the use of such spirometers (1 3) The aim of this study was to compare two new electronic spirometers Drager Spirotron and Monaghan M403 and one wedge bellows spirometer Vitalograph with the Bernstein spirometer and the Wright peak flow meter on healthy children

METHODS

Four spirometers were used

1 A modified Bernstein spirometer (?)
2 Vitalograph which is a wedge bellows spirometer
3 Spirotron (Drager) which is a pressure triggered spirometer The changes of pressure are electronically translated to lung volumes and maximal expiratory rates of the flow (PEFR)
4 Monaghan M403 in which a heated thermistor is cooled by the exhaled air The hanges of temperature are electronically translated to lung volumes and PEFR The back flow valve of the apparatus was not u ed

The Wright peak flow meter (for adults) was also used

The Monaghan equipment was calibrated against the Bernstein spirometer so that the value given by the Monaghan spirometer would correspond to the ATPS value of the Bernstein The original calibrations of the Spirotron and the Vitalograph were used The values were regarded to correspond to the ATPS values of the Bernstein spirometer

Each hild was investigated with all spirometers on the same oc asion and according to a rotatory schedule

All the tests were done with the subject in a sitting position Three values were taken each time of which the highest was chosen

The Bernstein spirometry was performed by a trained laboratory nurse and the other investigation by two paediatricians (the authors)

The Spirotron the Monaghan and the Vitalograph give the forced vital capacity (FVC) Two-stage vital capacity (VC) was done in the Bernstein spirometry but if the FVC

Table 1 *Vital capacity (VC) and forced expiratory volume in one second ($FEV_{1.0}$) of four spirometers for the height 130 cm 145 cm and 160 cm*

Capacity and volume in liters

	Height					
	130 cm		145 cm		160 cm	
Spirometer	VC	$FEV_{1.0}$	VC	$FEV_{1.0}$	VC	$FEV_{1.0}$
Bernstein	1 74	1 51	2 41	2 09	3 24	2 81
Spirotron	1 69	1 50	2 42	2 10	3 33	2 85
Monaghan	1 70	1 52	2 44	2 10	3 36	2 81
Vitalograph	1 72	1 57	2 44	2 16	3 33	2 90

value obtained during the maneuver of forced expiratory volume in one second ($FEV_{1.0}$) exceeded the best two stage VC value the FVC value was used In the following presentation the term VC is used for both VC and FVC

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30 girls and 31 boys took part in the testing Their age range was 7–15 years (mean 10 8 years) and their height range was 119–180 cm (mean 145 cm)

The following criteria of selection were used

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2 An interview by telephone with the parents served to eliminate any suspicion of previous or present serious disease previous or present pneumonia or bronchial obstruction
3 A routine physical examination and a renewed interview at the time of investigation served to disclose other abnormalities

About ten children selected according to criterium one were excluded according to criteria two and three and were replaced by other children For technical reasons the investigation with the Monaghan spirometer was only performed on the first 50 children

RESULTS

As seen from the regression lines of VC and $FEV_{1.0}$ (Figs 1 and 2) and from the examples

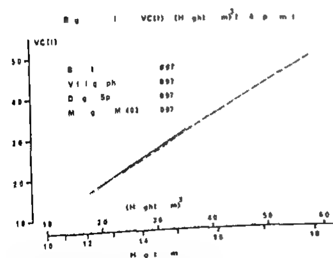


Fig 1 Vital capacity (VC) expressed in l in relation to body height to the third power (H^3) The equations of the regression lines are

VC (Bernstein) $=0\ 7895\times H^3+0\ 0013$ S D $=0\ 281$
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VC (Monaghan) $=0\ 8783\times H^3-0\ 2342$ S D $=0\ 281$
VC (Vitalograph)$=0\ 8458\times H^3-0\ 1340$ S D $=0\ 261$

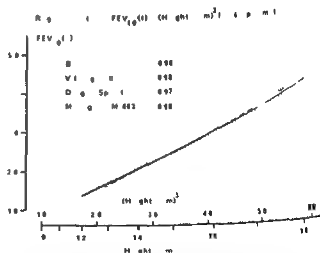


Fig 2 Forced expiratory volume in one second ($FEV_{1.0}$) expressed in l in relation to body height in the third power (H^3) The equations of the regression lines are

FEV_1 (Bernstein) $=0\ 6829\times H^3+0\ 0123$ S D $=0\ 211$
$FEV_{1.0}$ (Spirotron) $=0\ 7117\times H^3-0\ 0664$ S D $=0\ 221$
$FEV_{1.0}$ (Monaghan) $=0\ 6782\times H^3+0\ 0299$ S D $=0\ 201$
$FEV_{1.0}$ (Vitalograph)$=0\ 7010\times H^3+0\ 0256$ S D $=0\ 211$

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The differences are Bernstein minus Spirotron (B–S) Bernstein minus Monaghan (B–M) Bernstein minus Vitalograph (B–V)

	M (l)		S D (l)	
	VC	FEV_1	VC	FEV_1
B–S	−0 0?	−0 01	0 16	0 13
B–M	−0 07[a]	−0 03	0 16	0 1?
B–V	−0 04[a]	−0 07	0 11	0 10

Not significant [a] $p<0.01$ $p<0.001$

given in Table 1 the differences between the values obtained by the Bernstein spirometer and those by the other ones are very small

A nonparametric permittation test for the paired differences showed significancies for the means of VC of the Vitalograph and of the Monaghan (Table 2) Significance was also found for the mean difference of $FEV_{1.0}$ of the Vitalograph The mean differences are how ever small

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$FEV_{1.0}$ as a percentage of VC (FEV%) is also very similar for the different pieces of equipment (Table 3)

The differences of PEFR are substantial and highly significant (Table 4)

DISCUSSION

Previous studies of the reliability of electronic spirometers have given different results (1 3 5)

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Bernstein	87	76
Spirotron	87	75
Monaghan	86	75
Vitalograph	89	79

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	Mean (l/min)
W–S	−65
W–S_2	−87
W–M	+35
W–M_2	+32

$p<0.001$

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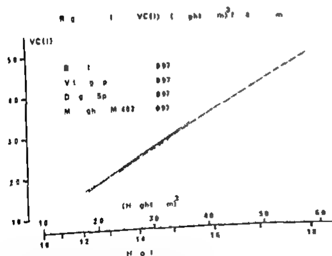


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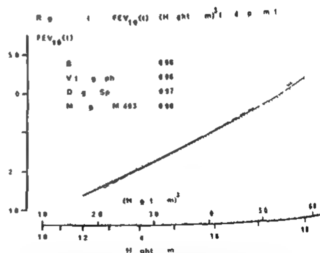


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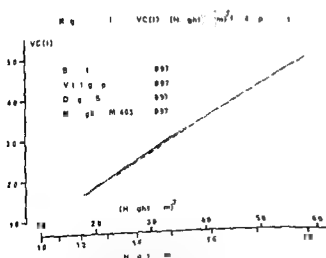


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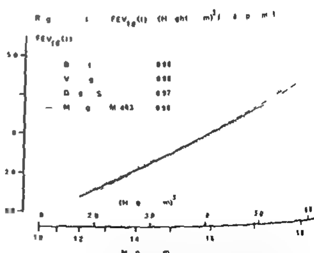


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RESULTS

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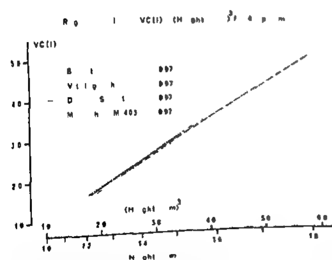


Fig 1 Vital capacity (VC) expressed in l in relation to body height to the third power (H^3) The equations of the regression lines are
VC (Bernstein) $=0.7895\times H^3+0.0013$ S D $=0.281$
VC (Spirotron) $=0.8623\times H^3-0.1340$ S D $=0.281$
VC (Monaghan) $=0.8783\times H^3-0.2342$ S D $=0.281$
VC (Vitalograph) $=0.8458\times H^3-0.1340$ S D $=0.261$

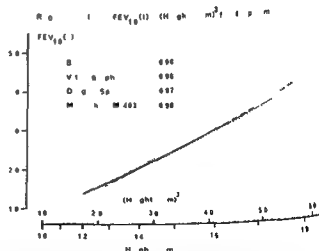


Fig 2 Forced expiratory volume in one second ($FEV_{1.0}$) expressed in l in relation to body height to the third power (H^3) The equations of the regression lines are
$FEV_{1.0}$ (Bernstein) $=0.68[illegible]9\times H^3+0.01[illegible]3$ S D $=0.211$
$FEV_{1.0}$ (Spirotron) $=0.7117\times H^3-0.0664$ S D $=0.[illegible]1$
$FEV_{1.0}$ (Monaghan) $=0.6792\times H^3+0.0299$ S D $=0.[illegible]01$
$FEV_{1.0}$ (Vitalograph) $=0.7010\times H^3+0.0256$ S D $=0.211$

Table 2 *Mean (M) and standard deviation (S D) of paired differences of vital capacity (VC) and forced expiratory volume in one sec ond ($FEV_{1\,0}$) obtained by four spirometers*

The differences are Bernstein minus Spirotron (B−S) Bernstein minus Monaghan (B−M) Bernstein minus Vitalograph (B−V)

	M (l)		S D (l)	
	VC	FEV	VC	FEV •
B−S	−0 0	−0 01	0 16	0 13
B−M	−0 07[b]	0 03	0 16	0 12
B−V	−0 04[b]	0 07	0 11	0 10

Not significant [b] $p<0\,01$ $p<0\,001$

given in Table 1 the differences between the values obtained by the Bernstein spirometer and those by the other ones are very small

A nonparametric permittation test for the paired differences showed significancies for the means of VC of the Vitalograph and of the Monaghan (Table 2) Significance was also found for the mean difference of $FEV_{1\,0}$ of the Vitalograph The mean differences are however small

The correlation coefficients of the regression lines are high for all the spirometers and the S D values around the lines are very similar (Figs 1 and 2)

$FEV_{1\,0}$ as a percentage of VC (FEV%) is also very similar for the different pieces of equipment (Table 3)

The differences of PEFR are substantial and highly significant (Table 4)

DISCUSSION

Previous studies of the reliability of electronic spirometers have given different results (1 3 5)

A mechanical model study showed some inadequacies of accuracy e g alinearity (5) In the same study a clinical investigation of adults showed a deviation of ±20% *from the* water spirometer on some subjects The mean values were however close to those of the water spirometer Our S D values of the

Table 3 *Forced expiratory volume in one sec ond as a percentage of vital capacity (FEV %) for four spirometers*

Mean (*M*) and the lower normal limit (*M*−2 S D) are given

Spirometer	*M*	*M*−2 S D
Bernstein	87	76
Spirotron	87	75
Monaghan	86	75
Vitalograph	89	79

paired differences of VC (0 16 l) and of $FEV_{1\,0}$ (0 12 l) are 6 6% and 5 7% respectively of the mean VC value and the mean $FEV_{1\,0}$ value obtained by the Bernstein spirometer (Table 2) If the mean differences are included then the limits of the *total* deviation (mean deviation of paired differences ±2 S D of paired differences) are +16% and −10% of the mean Bernstein VC value For the Vitalograph the limits of total deviation from the Bernstein value are less

The deviations include not only differences in the equipment (e g errors of calibrations) but also possible differences in the performance of the laboratory nurse (water spirometer) and the two paediatricians (the other spirometers) and possible psychological and biological changes of the children from one investigation to the other With one and the same laboratory nurse Engstrom et al (4)

Table 4 *Mean of paired differences of peak expiratory flow rate (PEFR) obtained by Wright s peak flow meter (W) Spirotron (S) and Monaghan (M)*

S_1 and M_1 denote the values obtained during the procedure of forced vital capacity *S* and *M* denote the values obtained from a separate PEFR procedure

	Mean (l/min)
W−*S*	−65
W−*S*	−87
W−*M*	+35
W−M_1	+3

$p<0\,001$

Table 1 *Vital capacity (VC) and forced expiratory volume in one second ($FEV_{1.0}$) of four spirometers for the height 130 cm 145 cm and 160 cm*

Capacity and volume in liters

	Height					
	130 cm		145 cm		160 cm	
Spirometer	VC	$FEV_{1.0}$	VC	$FEV_{1.0}$	VC	$FEV_{1.0}$
Bernstein	1 74	1 51	2 41	2 09	3 24	2 81
Spirotron	1 69	1 50	2 42	2 10	3 33	2 85
Monaghan	1 70	1 52	2 44	2 10	3 36	2 81
Vitalograph	1 72	1 57	2 44	2 16	3 33	2 90

value obtained during the maneuver of forced expiratory volume in one second ($FEV_{1.0}$) exceeded the best two-stage VC value the FVC value was used In the following presentation the term VC is used for both VC and FVC

MATERIAL

30 girls and 31 boys took part in the testing Their age range was 7–15 years (mean 10 8 years) and their height range was 119–160 cm (mean 145 cm)

The following criteria of selection were used

1 Seven children from each of grades one to nine of an ordinary school were selected by the school nurse Their health was verified by the school health records
2 An interview by telephone with the parents served to eliminate any suspicion of previous or present serious disease previous or present pneumonia or bronchial obstruction
3 A routine physical examination and a renewed interview at the time of investigation served to disclose other abnormalities

About ten children selected according to criterium one were excluded according to criteria two and three and were replaced by other children For technical reasons the investigation with the Monaghan spirometer was only performed on the first 50 children

RESULTS

As seen from the regression lines of VC and $FEV_{1.0}$ (Figs 1 and 2) and from the examples

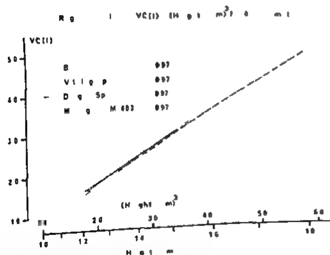


Fig 1 Vital capacity (VC) expressed in l in relation to body height to the third power (H^3) The equations of the regression lines are
VC (Bernstein) $=0\ 7895\times H^3+0\ 0013$ S D $=0\ 28$ l
VC (Spirotron) $=0\ 8623\times H^3-0\ 1340$ S D $=0\ 28$ l
VC (Monaghan) $=0\ 8783\times H^3-0\ 2342$ S D $=0\ 28$ l
VC (Vitalograph) $=0\ 8458\times H^3-0\ 1340$ S D $=0\ 26$ l

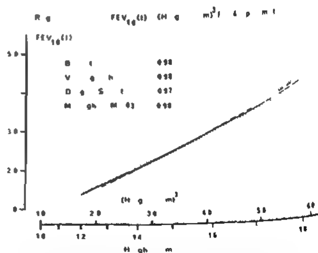


Fig 2 Forced expiratory volume in one second ($FEV_{1.0}$) expressed in l in relation to body height to the third power (H^3) The equations of the regression lines are
FEV_1 (Bernstein) $=0\ 6929\times H^3+0\ 0123$ S D $=0\ 21$ l
FEV_1 (Spirotron) $=0\ 7117\times H^3-0\ 0664$ S D $=0\ 21$ l
$FEV_{1.0}$ (Monaghan) $=0\ 6782\times H^3+0\ 0299$ S D $=0\ 01$
FEV_1 (Vitalograph) $=0\ 7010\times H^3+0\ 0256$ S D $=0\ 21$ l

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SERUM FERRITIN IN ASSESSMENT OF IRON NUTRITION IN HEALTHY INFANTS

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ABSTRACT Saarinen U M and Siimes M A (Children s Hospital University of Helsinki Finland) Serum ferritin in assessment of iron nutrition in healthy infants Acta Paediatr Scand 67 745 1978 —We followed up 238 infants on 7 occasions during their first year of life The diets of the infants were systematically either supplemented or not supplemented with iron Developmental changes in serum ferritin were determined from a group with adequate intake of iron and without evidence of iron deficiency by three laboratory criteria hemoglobin mean corpuscular volume and transferrin saturation The data indicate that the average level of serum ferritin correlates well with iron nutrition within groups of infants since the developmental changes are in accordance with the known changes in storage iron the level of serum ferritin correlates with iron intake and low ferritin levels are associated with lower transferrin saturation The usefulness of serum ferritin as the sole criterion of iron deficiency in individual infants is limited suggesting the use of more than one indicator to refine the diagnosis of iron deficiency without anemia

KEY WORDS Ferritin infant nutrition iron

The assessment of body storage iron by serum ferritin is a valid and simple method based on several observations in adults The concentration of serum ferritin is lower in females than in males in accordance with the differences in the storage iron (4 8) Low values are found in groups characterized by iron deficiency anemia whereas extremely high values are mainly associated with iron overload (1 11 14) In patients with transfusion siderosis and haemochromatosis the amount of storage iron can be approximated by the level of serum ferritin (11) with some exceptions A number of various diseases seem to result in an inappropriate increase in serum ferritin (6 14 22) whereas iron deficiency anemia is the only known condition associated with abnormally low levels

Practically every conclusion of the value of serum ferritin in the assessment of iron stores is based on results obtained from groups of subjects In fact there seems to be a good correlation (1 4 8 11 14 21) However less data are available about the correlation at the individual level especially within the normal range of values Repeated venesections of three normal men have resulted in rapid decline in the concentration of serum ferritin prior to the development of anemia (11) Particularly this observation initiated the hypothesis of the serum ferritin concentration as a sensitive detector of early iron deficiency However the marked developmental changes especially in infancy (21) may further modify the diagnostic validity of a single serum ferritin value

In the present study we took advantage of a follow up study of infants who were investigated on seven different occasions during their first year of life and whose diets were systematically either supplemented or not supplemented with iron The reference values were obtained from infants continuously supplemented with iron after exclusion of some infants suspected of iron deficiency by other laboratory criteria By means of these longitudinal data we wanted to approach the question whether a single serum ferritin value re

found the following S D of the differences from duplicate investigations with one and the same water spirometer 0 07 l (VC) 0 15 l ($FEV_{1.0}$) Considering all these facts we regard our S D values acceptable

As in the previous studies of adults (1 3 5) our mean values are close to those of the Bernstein spirometer (Table 2) A tendency to overestimation of the volumes was found for the Monaghan spirometer and for the Vitalograph which agrees with the results of Cox et al (3) but differences are fairly small The systematic higher mean value of VC for the Monaghan spirometer (0 07 l) might imply that the children cooperated better in the Monaghan procedure Another possible explanation is that the backflow valve was not used The Monaghan spirometer has the disadvantage that the digitally read out values will disappear or be erroneously high if the subject after the FVC maneuver begins to inhale before disconnection from the mouthpiece A backflow valve offered by the manufacturer prevents this error The close agreement of the mean $FEV_{1.0}$ values of the two spirometers supports this explanation

The correlation coefficients of the regression lines and the S D values around the lines are as good for the simple spirometers as for the Bernstein one (Figs 1 and 2)

A substantial and highly significant difference was found between the PEFR values of the electronic spirometers and the values of the Wright peak flow meter (Table 4) PEFR values obtained by the electronic spirometers are thus not comparable with those of the Wright peak flow meter

The Spirotron and the Vitalograph lack simple means of calibration The calibration facility of the Monaghan spirometer presents only little difficulty if a water spirometer is available for comparison We are however uncertain about the accuracy of the calibration The fact that our Vitalograph has been in use for 5 years seems to justify the original calibration and has given us an impression of this simple device as being reliable Hygienic arguments are however often raised against the bellows spirometers which are said to collect mould

The electronic spirometers and the Vitalograph have many advantages compactness simple operation and fast delivery of data which are necessary for an extensive use during functional investigations on children with pulmonary disease Our results show a fairly good reliability on healthy children even though further studies are needed

Most important of all is that the equipment must be evaluated on children suffering of bronchial asthma which is the most common serious pulmonary disease during childhood

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Table 1 *Serum ferritin normal values (µg/l) in infants*

The samples were obtained from infants on prolonged iron supplementation and with iron deficiency excluded by laboratory criteria (Hgb below 110 g/l MCV below 71 fl S% below 10%) and from cord blood

	Age (months)							
	0	0 5	1	2	4	6	9	12
n	98	46	46	47	40	36	43	42
+2 S D	395	678	399	430	223	147	103	91
Mean	160	238	240	194	91	51	39	31
− S D	65	90	144	87	37	19	14	11

below the age specific means The lower limit gradually decreased to 11 µg/l by the age of one year (Table 1)

The data further indicate some evidence of physiologic individuality with respect to the level of serum ferritin within the normal range of values in infants with sufficient iron intake An example of this phenomenon is demonstrated in Fig 1 by following infants with values above +1 S D and below −1 S D of the initial range When the two groups were made up at different ages and subsequently compared to each other their values remained significantly different ($p<0$ 01 in most cases) for a period of 5 to 7 months

On the other hand the level of cord blood ferritin seemed not to have prognostic value in the detection of future iron deficiency The Hgb MCV and S% of the infant groups with either high or low cord blood ferritin concentrations were compared at the ages of 6 9 and 12 months and no significant differences were noticed

Within infant groups the average level of serum ferritin correlated with iron nutrition The infants on prolonged iron supplementation had higher ferritin values than the nonsupplemented infants at the ages of 6 9 and 12 months ($p<0$ 005 $p<0$ 001 and $p<0$ 001 respectively) (Fig 2) In order to approach the question how accurately a single determination of the concentration of serum ferritin reflects the iron nutrition we followed up infants with and without iron supplementation during the latter half of infancy Of the infants with serum ferritin above +1 S D at 6 months of age the iron supplemented ones maintained their ferritin levels above the age specific mean (Fig 3) On the other hand the high ferritin values (above +1 S D) at 6 months of age decreased more than the developmental changes would allow in each infant without iron supplementation (Fig 3) Accordingly a nonoptimal iron nutrition is associated with decreasing levels of serum ferritin not only within groups of infants but also in individuals within the normal range

In the iron supplemented infants the low values of serum ferritin (below −1 S D) at 6 months of age remained within the lower range of normal through the next 6 months as one

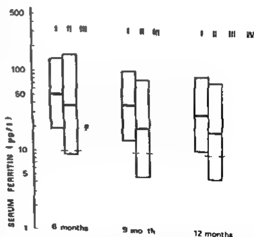


Fig 2 I Serum ferritin values of iron supplemented infants (n=36–43) II Nonsupplemented infants (n=115) III Infants with two simultaneous criteria of iron deficiency (Hgb MCV or S%) IV Infants with severe iron deficiency anemia The means with ±2 S D ranges (I–II) or individual values (III IV) are shown

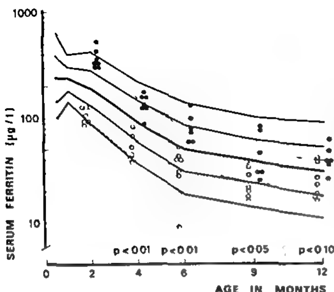


Fig 1 Serum ferritin values of high and low borderline groups (values above +1 S D and below −1 S D respectively) in iron supplemented infants followed from 2 through 12 months of age The median values of the groups are shown by broken lines The solid lines indicate the distribution of normal values shown in Table 1

flects the iron nutrition in individual infants or whether the ferritin level is useful in this respect only within groups of infants

SUBJECTS

The entire series consisted initially of 256 healthy newborns with birthweight over 3 0 kg and of gestational age between 38 and 42 weeks They were born at the Helsinki University Central Hospital and examined at the Children s Hospital at the ages of 2 weeks and 1 2 4 6 9 and 12 months A total of 238 infants 126 boys and 112 girls completed this period of follow up We also had the cord blood samples of [illegible] of these infants

At the time of weaning half of the infants chosen at random at the time of birth were started on home prepared cow s milk formula and the other half on proprietary infant milk formula (Bona® Chymos Oy Lappeenranta Finland) Weaning occurred gradually and 25% of the infants were still breast fed at 6 months of age The infants fed breast milk or cow s milk formula prepared at home received no iron supplementation whereas the proprietary formula was supplemented with 11 mg of elemental iron/l as ferrous gluconate The formula was continued through the first year of life Solid foods were introduced to all infants at 3 5 months of age the cereals were started at 5 months of age and were supplemented with iron for the proprietary formula fed infants

Study groups

In addition to the whole infant series two subgroups were also evaluated and designated as follows

A *Iron supplemented group* A total of 47 infants were weaned to the iron supplemented formula prior to the age of 1 month The amount of daily supplemental iron was expected to be sufficient to prevent iron deficiency during the first year of life (2) However a few infants were excluded owing to laboratory criteria of suspected iron deficiency 1) hemoglobin (Hgb) below 110 g/l (7 19) one infant at [illegible] months of age 2) mean corpuscular volume of red blood cells (MCV) below 71 fl (12 19) 3 1 and 1 infants at the ages of 6 9 and 12 months respectively 3) transferrin saturation (S%) below 10% (18) 3 4 1 and 1 infants at the ages of 4 6 9 and 12 months respectively Data on S% were lacking in 4 3 2 and 3 infants at the above mentioned ages These infants were also excluded

B *Nonsupplemented group* A group of 115 infants were weaned at different ages to the home prepared cow s milk formula and received no supplemental iron during their first year of life Breast feeding was common within this group and half of these infants were still breast fed at the age of 6 months

METHODS

At every visit about 2 5 ml of venous blood was drawn 1 ml into EDTA and 1 5 ml into an iron free tube The serum was removed and frozen within 2 to 3 hours

Serum ferritin was determined in triplicate by a radioimmunometric assay (21) Hemoglobin and red blood cell indices were determined by a Model S Coulter Counter The reticulocytes were counted microscopically Serum iron (SI) and total iron binding capacity (TIBC) were measured spectrophotometrically in duplicate (3 17) The transferrin saturation was calculated from the ratio SI/TIBC×100

Statistical analyses

The means and standard deviations as well as Student s *t* test were used in the statistical analyses The serum ferritin values had a normal distribution after logarithmic transformation Thus the analyses were performed using the logarithms In comparing values when a normal distribution was not expected (Fig 1 Table 2) the nonparametric U test of Mann and Whitney was used

RESULTS

The developmental changes of serum ferritin during the first year of life were obtained by the follow up of iron supplemented infants without evidence of iron deficiency by other laboratory criteria The concentrations were high at birth rose further during the first month of life and thereafter gradually decreased through infancy (Table 1) There were no sex differences The lower limits of normal were considered to equal the −2 S D levels

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	Age (months)							
	0	0 5	1	2	4	6	9	1
n	98	46	46	47	40	36	43	4?
+ S D	395	6?8	399	430	??3	14	103	91
Mean	160	?38	?40	194	91	51	39	31
−? S D	65	90	144	87	37	19	14	11

below the age specific means The lower limit gradually decreased to 11 μg/l by the age of one year (Table 1)

The data further indicate some evidence of physiologic individuality with respect to the level of serum ferritin within the normal range of values in infants with sufficient iron intake An example of this phenomenon is demonstrated in Fig 1 by following infants with values above +1 S D and below −1 S D of the initial range When the two groups were made up at different ages and subsequently compared to each other their values remained significantly different ($p<0\ 01$ in most cases) for a period of 5 to 7 months

On the other hand the level of cord blood ferritin seemed not to have prognostic value in the detection of future iron deficiency The Hgb MCV and S% of the infant groups with either high or low cord blood ferritin concentrations were compared at the ages of 6 9 and 12 months and no significant differences were noticed

Within infant groups the average level of serum ferritin correlated with iron nutrition The infants on prolonged iron supplementation had higher ferritin values than the nonsupplemented infants at the ages of 6 9 and 12 months ($p<0\ 005$ $p<0\ 001$ and $p<0\ 001$ respectively) (Fig 2) In order to approach the question how accurately a single determination of the concentration of serum ferritin reflects the iron nutrition we followed up infants with and without iron supplementation during the latter half of infancy Of the infants with serum ferritin above +1 S D at 6 months of age the iron supplemented ones maintained their ferritin levels above the age specific mean (Fig 3) On the other hand the high ferritin values (above +1 S D) at 6 months of age decreased more than the developmental changes would allow in each infant without iron supplementation (Fig 3) Accordingly a nonoptimal iron nutrition is associated with decreasing levels of serum ferritin not only within groups of infants but also in individuals within the normal range

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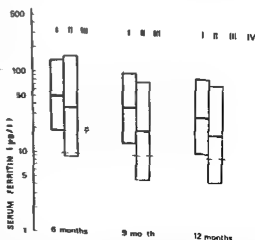


Fig 2 I Serum ferritin values of iron supplemented infants (n=36–43) II Nonsupplemented infants (n=115) III Infants with two simultaneous criteria of iron deficiency (Hgb MCV or S^{cr}) IV Infants with severe iron deficiency anemia The means with ±? S D ranges (I–II) or individual values (III–IV) are shown

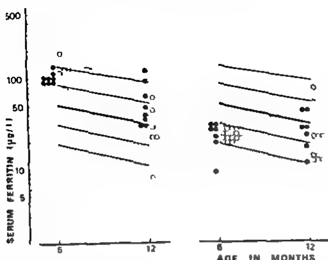


Fig 3 Serum ferritin values of the high (above +1 S D left) and low (below −1 S D right) borderline groups were followed from 6 to 12 months of age Open circles (between −1 S D and −2 S D) and open squares (below −2 S D) represent the nonsupplemented infants whereas the solid circles are infants on prolonged iron supplementation The broken lines show changes in the ranges of the values and the solid lines indicate the distribution of normal values similarly as in Fig 1

might expect and as previously noted from the observation of the individual levels Surprisingly in the respective nonsupplemented infants the ferritin values spread throughout the scale and only a proportion (27%) further decreased below −2 S D by 12 months of age (Fig 3)

We also tried to evaluate the association between the low values of serum ferritin and other independent criteria of iron nutrition such as Hgb MCV and S% One might expect to find either more abnormally low values or lower mean values of these parameters associated with low serum ferritin values if a ferritin value at the lower range of normal would reflect an amount of storage iron at the lower range In fact there was no difference either in Hgb MCV or S% at 6 or 12 months of age in iron supplemented infants with different levels of serum ferritin (Table 2) However in the nonsupplemented infants there was a correlation between serum ferritin and S% especially at 6 months of age (Table 2) The infants with serum ferritin below −3 S D had a significantly lower S% than the infants with serum ferritin above +1 S D ($p<0.02$) There was no such correlation regarding the mean values of Hgb or MCV

In order to further analyse the validity of serum ferritin as a screening test for mild iron deficiency in late infancy individual infants were selected who developed iron deficiency as defined by two simultaneously abnormal criteria (Hgb MCV or S%) Within the whole series of 238 infants there were 6 such infants at 6 months 3 at 9 months and 6 at 12 months of age (Table 3) The serum ferritin values of these infants were mostly within the normal range and only 5 of the values were below −2 S D of the age specific mean The serum ferritin values of these infants were plotted in Fig 2 in comparison with the ranges of values (mean ±2 S D) of iron supplemented infants nonsupplemented infants and infants with severe iron deficiency anemia (16) The last mentioned infants were treated for verified iron deficiency anemia at the Children s Hospital their Hgb values ranged from 50 to 78 g/l and their ages from 9 to 17 months

There were 6 out of the 238 infants who developed mild iron deficiency anemia as defined by laboratory criteria and the response to iron medication reticulocytosis and an increase in Hgb Two of these cases are shown in detail in Fig 4 in order to demonstrate the sequence of events in the natural development of mild iron deficiency anemia prior to and after iron treatment In the first patient the concentration of serum ferritin decreased considerably and dropped below the lower limit prior to the development of anemia (Fig 4) In the second patient the ferritin concentration was initially unusually high but also decreased although it still was above the normal range at the time anemia was evident (Fig 4) However common to both infants was a change to a lower ferritin channel compared to the normal developmental pattern This phenomenon was also found in 10 out of the 13 infants with two simultaneous criteria of iron deficiency

Table 2 *The transferrin saturation in infant groups with different serum ferritin levels*

The median values for S% are shown both for infants supplemented with iron and for those without iron supplementation ±1 S D ±2 S D and −3 S D represent the distribution of the serum ferritin normal values after log transformation (Table 1) and are the limits between which the infant groups were selected The values between +1 S D and −1 S D are omitted for the sake of clarity

At 6 months					At 12 months			
With iron		Without iron			With iron		Without iron	
n	S%	*n*	S%		*n*	S%	*n*	S%
				+2 S D				
8	28%	10	21%		7	18%	7	28%
				+1 S D				
				−1 S D				
7	6%	23	14%		7	24%	30	19%
				−2 S D				
1	6%	9	14%		–	–	10	0%
				−3 S D				
	–	5	10%		–	–	7	15%

p<0 0 by use of the nonparametric U test of Mann and Whitney

DISCUSSION

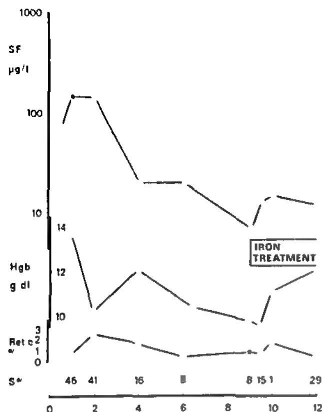
The developmental changes of serum ferritin values correlate well with the changes in body storage iron during infancy (20 23) In addition the lower limit of normal by one year of age is close to previous suggestions (21) The physiologic individuality of the serum ferritin values within the normal range was a new finding and suggests similar physiologic channels for serum ferritin as recently found for Hgb and red blood cell indices (19)

Serum ferritin proved to be a useful test in evaluating iron nutrition in groups of infants The level of serum ferritin correlated well with iron intake and low ferritin levels were associated with low transferrin saturation An unexpected finding was a rise of the serum ferritin values during the latter half of infancy in

Table 3 *Infants with two criteria of iron deficiency*

The criteria were Hgb below 110 g/l MCV below 71 fl and S% below 10%

No of infant (*n*=13)	Age (months)	Hgb (g/l)	MCV (fl)	S% (%)	Ferritin (µg/l)
118	[illegible]	109	74	8 5	21
138	[illegible]	1 1	68	8 7	19
4	6	104	72	8 0	0
84	[illegible]	1 5	66	9 6	[illegible]
105	6	118	67	5 7	39
113	6	109	71	8 3	118
143	9	119	69	9 4	7
4	9	98	6	8 1	7
145	9	101	78	8 5	74
39	(	1 3	70	7 4	61
37	12	109	68	10 8	36
84	1	11	64	9 7	32
66	1	105	73	7 2	4
60	1	1 9	70	9 0	3
31	1	109	69	-1 6	16



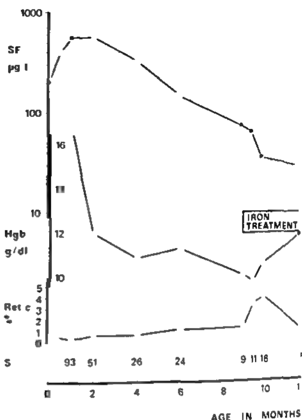


Fig. 4. Developmental changes of Hgb, S%, and serum ferritin in two individual infants who developed iron deficiency anemia at 9 months of age and showed response to iron treatment by reticulocytosis and rise in Hgb. The normal range of serum ferritin (mean ±2 S.D.) is indicated by dotted lines.

many of the infants not supplemented with iron and with low serum ferritin values at 6 months of age. One might speculate that the initially lower iron stores at 6 months of age and the subsequent follow up without supplementation could induce enhanced alimentary absorption of iron and through this compensation even improve the iron status in many infants. However, we want to emphasize that this explanation is highly hypothetical and based on other studies indicating that depleted iron stores can enhance the bioavailability of iron (10, 24).

At the individual level the significance of a single serum ferritin value seems to be limited during the period of rapid growth and developmental changes. In severe iron deficiency anemia a low serum ferritin concentration seems to be a rule and of value in the differential diagnosis of anemia. On the other hand, in milder cases the signs of iron deficiency may appear before body iron stores are exhausted and/or serum ferritin has reached a subnormal level, as recently shown in small preterm infants after birth (15). Thus the serum ferritin concentration alone cannot be regarded as a useful screening test for mild iron deficiency in infancy although it was expected according to the previous studies in adults and children.

An early change to a lower "ferritin channel" in the development of iron deficiency might be of academic interest, but hardly of any value in the routine diagnosis.

Although the usefulness of the serum ferritin concentration is limited as the sole criterion of iron deficiency in individual infants, the use of more than one indicator may well refine the diagnosis of iron deficiency without anemia even in infants (5, 9, 13). On the other hand, our data confirm the value of serum ferritin in the assessment of iron nutrition in infant

groups and in differential diagnosis of severe anemia

ACKNOWLEDGEMENTS

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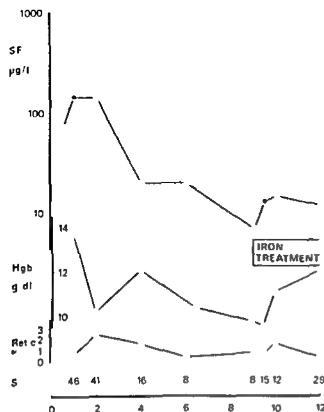
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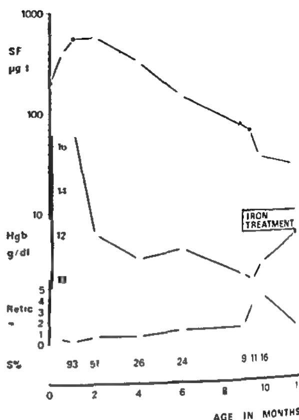


Fig. 4 Developmental changes of Hgb S% and serum ferritin in two individual infants who developed iron deficiency anemia at 9 months of age and showed response to iron treatment by reticulocytosis and rise in Hgb The normal range of serum ferritin (mean ±2 S D) is indicated by dotted lines

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DDAVP IN CHILDHOOD NOCTURNAL ENURESIS

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ABSTRACT. Tuvemo, T. (Department of Paediatrics, University Hospital, Uppsala, Sweden). DDAVP in childhood nocturnal enuresis. Acta Paediatr Scand, 67: 753, 1978.—A double-blind study of 18 children aged 6–12 years suffering from primary nocturnal enuresis without signs of underlying organic disease is reported. 20 µg of DDAVP (desamino-D-arginine vasopressin, Minirin®) was given intranasally at bedtime. The effect was prompt and satisfactory in 8 children and relatively good in another 8 children. No adverse effects were noted. DDAVP is advocated for temporary use in children with nocturnal enuresis needing immediate help.

KEY WORDS: School children, nocturnal enuresis, DDAVP

Enuresis nocturna is a frequent problem encountered in schoolchildren and may be the cause of much distress (10). Over the years a variety of regimes have been applied in the treatment of this condition (5, 7). In controlled studies two methods have proved to be of significant value, i.e. treatment with tricyclic antidepressant drugs, e.g. imipramine (Tofranil®) (3, 4, 9) and the application of various conditioning devices (4, 6). When properly used both alternatives have yielded good results (3, 5). Especially when using tricyclic antidepressants, however, side effects occur (5, 8), some of serious import (8). Furthermore, the effect of the treatment is seldom prompt but tends to show a lag period of a week or more. Using both methods of treatment, Kolvin et al. (4) found maximum effect during the second month of treatment, but the effect of conditioning devices may occur even later (10).

Demand for effective treatment of enuresis nocturna may arise with short notice, e.g. on unexpected journeys, attending camp, etc., all events of great social importance in the child. None of the above mentioned methods of treatment satisfy such demands. Since the administration of desamino-D-arginine vasopressin (DDAVP) to six bed wetting children in similar social situations had yielded promising results, it was decided to investigate the effect of the drug on enuresis nocturna in a double blind study.

METHOD

Children of at least 6 years of age suffering from primary nocturnal enuresis were chosen for the study. The children had not responded satisfactorily to previous treatment with imipramin (Tofranil®) or amitriptylin (Tryptizol®) 1–2 mg/kg for ≥28 days. The group consisted of 18 children aged 6–12 years, 9 of which had a family history of enuresis. A careful case history and physical examination as well as analysis of the urine including bacterial cultures were performed in each case. There was no suspicion of any organic disease in the group studied. Each patient was given a placebo for 28 days and DDAVP (desamino-D-arginine vasopressin, Minirin®, Ferring AB, Sweden) for 28 days in a daily dose of 20 µg, administered intranasally. The study was randomized and the code was unknown to both patient and investigator. The patients were unable to distinguish placebo from drug by smell, taste or the like. The patients were instructed to empty their bladder and take the drug just before bedtime. The number of dry nights were counted during both 28-day periods as well as during a 28-day period prior to the study. When the code was broken it was found that 8 patients had received DDAVP during the first period of 28 days, the remaining 10 patients during the second 28-day period. Before and after the periods of treatment hemoglobin (Hb), sedimentation rate (MSR), serum creatinine, serum sodium (S-Na) and blood pressure (BP) were measured and routine physical examinations were performed. A careful history was taken regarding the personal experience of the treatment.

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KEY WORDS School children nocturnal enuresis DDAVP

Enuresis nocturna is a frequent problem encountered in schoolchildren and may be the cause of much distress (10) Over the years a variety of regimes have been applied in the treatment of this condition (5 7) In controlled studies two methods have proved to be of significant value i e treatment with tricyclic antidepressant drugs e g imipramine (Tofranil®) (3 4 9) and the application of various conditioning devices (4 6) When properly used both alternatives have yielded good results (3 5) Especially when using tricyclic antidepressants however side effects occur (5 8) some of serious import (8) Furthermore the effect of the treatment is seldom prompt but tends to show a lag period of a week or more Using both methods of treatment Kolvin et al (4) found maximum effect during the second month of treatment but the effect of conditioning devices may occur even later (10)

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Children of at least 6 years of age suffering from primary nocturnal enuresis were chosen for the study The children had not responded satisfactorily to previous treatment with impipramin (Tofranil®) or amitriptylin (Tryptizol®) 1–2 mg/kg for ≥ 8 days The group consisted of 18 children aged 6–12 years 9 of which had a family history of enuresis A careful case history and physical examination as well as analysis of the urine including bacterial cultures were performed in each case There was no suspicion of any organic disease in the group studied Each patient was given a placebo for 28 days and DDAVP (desamino-D-arginine vasopressin Minirin® Ferring AB Sweden) for 28 days in a daily dose of 20 μg administered intranasally The study was randomized and the code was unknown to both patient and investigator The patients were unable to distinguish placebo from drug by smell taste or the like The patients were instructed to empty their bladder and take the drug just before bedtime The number of dry nights were counted during both 28 day periods as well as during a 28-day period prior to the study When the code was broken it was found that 8 patients had received DDAVP during the first period of 28 days the remaining 10 patients during the second 28-day period Before and after the periods of treatment hemoglobin (Hb) sedimentation rate (MSR) serum creatinine serum sodium (S Na) and blood pressure (BP) were measured and routine physical examinations were performed A careful history was taken regarding the personal experience of the treatment

Table 1. *Routine laboratory data and blood pressure before and after treatment*

Means ± S.D. are given

	Before treatment	After treatment
Hb (g/l)	127.8±7.9	127.5±7.8
MSR (mm)	10.6±7.4	9.5±5.6
S creat (μmol/l)	53.0±6.7	53.0±7.5
S Na (mmol/l)	142.5±3.1	140.5±1.5
BP[illegible] (mmHg)	114.0±8.0	111.0±8.0
BP[illegible] (mmHg)	76.0±6.0	74.0±6.0

RESULTS

All 18 children experienced more dry nights during the DDAVP period than during the placebo period. During DDAVP treatment the children were dry 22 (M=21.7, S.E.M. =1.72) out of 28 nights, while in the placebo period the mean value was 12 dry nights (M=12.1, S.E.M. =2.07). The difference is highly significant (p<0.001, Student's t test). In the period prior to the trial the children had been dry 8 (M=7.5, S.E.M. =2.98) out of 28 nights. The results can be described as excellent in 8 of the 18 children (27 or 28 dry nights out of 28), relatively good in 8 of the children (16–26 dry nights out of 28) and unsatisfactory in 2 children (5 dry nights out of 28).

A quantitative estimation of the volume of urine at enuresis was not attempted. Some mothers spontaneously reported that in cases where DDAVP had failed to prevent enuresis urine volumes were still considerably smaller than usual. No physical or subjective side effects of the treatment were observed. No significant changes in Hb, MSR or S creatinine were found during drug administration (Table 1).

In 3 patients S Na before the trial was found to be higher than the normal reference used (134–146 mmol/l). After the trial S Na values were found to be normal. After the trial BP values were slightly lower than before (p<0.1). These observations were regarded as irrelevant.

After the study most of the patients with good therapeutic results wanted to continue DDAVP treatment as they soon found that withdrawal of the drug produced relapse of bed wetting. In the children who experienced a relatively good but not satisfactory effect of DDAVP a higher dose (30–40 μg) was tried for a short period of time. This caused further improvement in some of the children.

DISCUSSION

This double blind study shows that DDAVP (20 μg) administered intranasally at bedtime was significantly superior to a placebo in preventing bed wetting. The mechanism is probably a reduction in urine volume keeping it within the limit of the bladder capacity of the child. In more than half of the children in this selected group of non antidepressant responders the effect of the dose used was satisfactory for practical purposes. The drug therefore enables many children to avoid bed wetting especially during nights when it may be of special importance to keep dry. The dose of the drug could not be related to the size of the child because of the double blind technique used. Optimal dosage has to be clarified in further studies. In some children more than 20 μg seems indicated. The long term effects have not been evaluated. This is an expensive drug, so long term treatment in large populations cannot be justified. For long term treatment a conditioning device is probably the regime of choice (4). As this method is unsatisfactory when rapid help is needed, DDAVP seems to be a valuable complement. The toxicity of DDAVP is reported to be very low and sensitization to DDAVP has not been reported. As in many other investigations (1, 2) no side effects of DDAVP were observed. Negative aspects of the treatment are the price of the drug and that some loss may occur on dispensing the solution.

In conclusion, DDAVP constitutes a valuable contribution to the therapeutic arsenal used in the treatment of enuresis nocturna without underlying organic disease. The drug

ıs partıculary useful for lımıted perıods of tıme when the patıent needs help wıth such short notıce that for ınstance condıtıonıng devıces cannot be applıed

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ADDENDUM

Sınce thıs materıal was collected Dımson has publıshed a pılot study (*Lancet* June 11 1977) on desmopressın as a treatment for enuresıs Hıs results agree quıte well wıth those of the present controlled study

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SOMATOMEDIN IN NEWBORNS AND THE RELATIONSHIP TO HUMAN CHORIONIC SOMATOTROPIN AND FETAL GROWTH

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ABSTRACT Kastrup K W Andersen H J and Lebech P (University Clinic of Paediatrics Childrens Hospital Fuglebakken and Department of Gynecology and Obstetrics Frederiksberg Hospital Copenhagen Denmark) Somatomedin in newborns and the relationship to human chorionic somatotropin and fetal growth Acta Paediatr Scand 67 757 1978 —The significance of somatomedin A (SM) and human chorionic somatomammotropin (HCS) in fetal growth was examined SM determined by chick embryo assay was studied during the last trimester of pregnancy in maternal serum and cord blood at term and in a group of normal newborns in the first week of life Furthermore a group of newborns of diabetic mothers was studied in the first or second day of life HCS was measured in maternal serum and in cord blood at term In amniotic fluid inhibitory factors caused a low SM activity as measured by the bioassay The following results were obtained 1) Normal values of SM in the last trimester with a decline at term were found in 3 normal primigravidae 2) The mean levels of SM in 22 mothers and their offspring were decreased The difference between the two values was significant but a positive correlation was found between the maternally related pairs of SM values Moreover a positive correlation was found between maternal SM birth weight and length HCS was not correlated to above mentioned parameters but there was positive correlation between placental weight and birth weight 3) In 6 newborns during the first 5 days SM rose from very low values to normal values found in infants in their first year 4) The mean value of SM in ten newborns of diabetic mothers was not significantly different from the mean value of control group The results do not exclude the possibility of a transplacental transport of SM and the positive correlation between SM levels and birth weight found in this investigation supports the concept that SM plays an important role in fetal growth

KEY WORDS Somatomedin human chorionic somatotropin newborn infants fetal growth

The nature of hormonal regulation important in fetal growth has not yet been clarified Although somatotropin (hGH) exercises a decisive influence on postnatal growth it appears to be of minor importance in fetal growth (10 15) The synthesis in the syncytiotrophoblast of chorion somatomammotropin (HCS) which is structurally closely related to somatotropin is increased during pregnancy but its somatotropic capacity is negligible whereas its lipolytic effect provides adequate amounts of free fatty acids and glucose during fetal growth (22)

In spite of the many investigations which have demonstrated a relationship between hGH and somatomedin (SM) none have detected a correlation between HCS and SM While trophic regulation by HCS of SM secretion which in turn plays an important role in fetal growth is an attractive hypothesis no clear evidence of somatomedin s influence in fetal growth has yet been found Earlier work has examined the relationship between SM levels in the mother and newborn infant as well as variations in SM during the first few days of life (1 2 8 14 16 21) and in cord

Table 1 *Somatomedin (U/ml) in last trimester and at term in normal pregnancy and in cord blood*

Week of gestation	SM (M±S D)
28	0 74±0 13
32	0 95±0 19
36	0 98±0 20
Term	0 66±0 21
Cord	0 61±0 18 (n=3)

blood alone (6 9) but the results obtained were equivocal and the state of affairs remained unresolved

Further examination of the problem was therefore carried out in the present investigation in an effort to establish a possible relationship between SM and fetal growth

To achieve this objective we analysed SM in the mother and in the umbilical cord blood of the newborn and compared these values with the HCS level in the mother and with the placental weight In addition we made consecutive SM analyses in a group of normal newborns during the first 6 days of life as well as in a group of newborns of diabetic mothers to assess the possible relationship between SM activity and the increased birth weight observed in those infants

MATERIAL AND METHODS

Somatomedin A modification of Hall s method of analysis of sulphate incorporation in the pelvic cartilage of 12 day old chick embryos was utilized (11) Incubation was carried out for 18 hours in Eagle s medium with essential amino acids and glutamine A pool of serum from adult non pregnant women served as a reference Serum concentrations of 7 5% to 30% were employed in a 4 point assay and quadruple determinations The data were treated in a program worked out by one of the authors (K W K) The quotients of variation for parallelity were tested at the 5% significance level By means of this method some assays were found to be not valid most often due to inhibitory activity in cord sera whereas most cord sera did not show inhibitory activity The interassay variation was about 15% and the mean precision index (lambda) for twenty consecutive determinations was 0 15±0 07

Human chorion somatotropin This hormone was determined by the radioimmunoassay method described by Lebech & Borggaard (17)

The material 1) 3 women in their first pregnancy who experienced no complications during pregnancy Blood samples were taken at the beginning of each of the last three months of pregnancy and immediately after parturition at which time a blood sample was also taken from the umbilical cord for SM analysis

2) 22 mothers and their newborns who were mature and born after normal pregnancies Blood samples for HCS and SM analyses were withdrawn immediately after parturition centrifuged and frozen for determination at a later time

3) After securing permission from their mothers venous blood samples were withdrawn from six newborns mature infants without perinatal complications during their first five days of life The first sample was taken between 6-18 hours after birth and the remainder at 24 hours intervals

4) Venous blood samples were taken in the first and second day of life from 10 infants of diabetic mothers and from 9 mature infants with mild perinatal complications

5) In three mature newborns in whom catheters were implanted for reasons of intensive care in both the umbilical artery and vein no difference was found between the SM values measured in the umbilical circulation from the value recorded from the peripheral vein and the venous blood value is considered to be representative of the body SM activity

RESULTS

SM during pregnancy In order to detect a possible variation in SM activity during pregnancy SM determinations of 3 first time pregnant women were carried out in the last trimester The results appear in Table 1 It can be seen that the average value of SM was normal in that period of pregnancy but was lower than normal at term Furthermore the SM value in the cord blood of the infants was

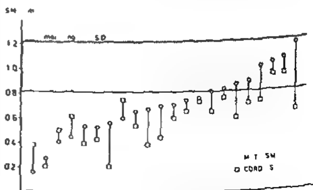


Fig 1 Somatomedin in maternal serum and in corresponding cord blood sample arranged according to increasing values in maternal serum

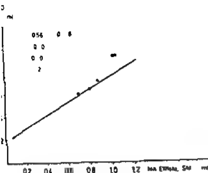


Fig 2 Somatomedin in maternal serum in relation to the corresponding cord blood sample

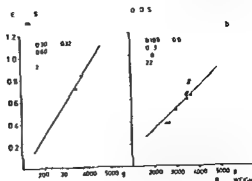


Fig 3 Somatomedin in maternal serum (*a*) and cord blood (*b*) in relation to birth weight

found to be located in the same concentration range as its mother s and there appeared to be a relationship between the magnitudes of the two values That observation led to the investigation described below

SM activities in maternal and umbilical cord blood HCS and SM determinations were carried out on blood samples obtained at parturition from 22 mothers and from the umbilical cords of their infants The results of the SM determinations are shown in Fig 1 The mean value of SM for the mothers was 0 70±0 25 U/ml and for the infant s cord blood 0 58±0 70 U/ml The difference between these two values was significant ($p<0\ 01$) A positive correlation was found between the weight of placenta and the birth weight but not between HCS and the above mentioned parameters Table 2 summarizes the coefficients of correlation and levels of significance referred to above There was a positive correlation between SM in mother and child at birth On paired examination the values of the children were lower than those of their mothers This relationship was confirmed by calculation of the correlation between maternally related pairs of SM values which was significantly positive (Fig 2)

In addition a comparison was made between the birth weight the mother s SM and the umbilical cord SM (Fig 3) A significant positive correlation was found in both cases Moreover a positive correlation was also found between the mother s SM and the infant s length at birth but not between the umbilical cord SM and infant s length at birth

The mean value of HCS in maternal serum was 6 6±2 2 μg/ml and in cord blood 0 02±0 01 μg/ml No correlation was found to SM in mothers or newborns As expected the magnitude of HCS values in umbilical cord blood were only about 1/100 of the maternal values and therefore no statistical calculations were performed

SM in the newborns Venous blood samples were withdrawn daily for the first five days of life from six normal newborn infants for SM determinations The mean value for the first day was 0 28±0 09 U/ml but it rose to 0 79±0 19 U/ml on the fifth day (Fig 4)

SM in infants of diabetic mothers Ten infants average weight 3 468 g born to closely

Table 2 *Correlation coefficients and levels of significance*

Parameters	*r*	*p*
Maternal SM vs placental weight	0 19	n s
Maternal SM vs birth weight	0 60	<0 01
Maternal SM vs birth length	0 79	<0 01
Cord SM vs placental weight	0 18	n s
Cord SM vs birth weight	0 44	<0 05
Cord SM vs birth length	0 61	n s
Cord SM vs maternal SM	0 70	<0 001
Placental weight vs birth weight	0 64	<0 05

Table 3. *Previous and present studies of somatomedin in cord blood and maternal blood*

Author	Cord SM U/ml ± S.D.	*n*	Maternal SM U/ml ± S.D.	*n*	Assay method	Ratio mat. SM/ cord SM
Chesley (2)	0.88	6	0.97	6	Costal cartilage hypophysectomized rats	1.10
D'Ercole et al. (6)	0.33±0.01	25			Placental membrane receptor	
Giordano et al. (8)	0.33±0.23	6	0.89±0.17	6	Costal cartilage intact rats	2.70
Gluckman et al. (9)	0.65±0.21	63			Porcine cartilage	
Hintz et al. (14)	0.50±0.19	52	0.41±0.20	28	Porcine cartilage	0.82
Swan et al. (20)	0.55±0.05	12	0.70±0.08	12	Radioreceptor	1.27
Tato et al. (21)	0.54±0.33	14	0.26±0.19	14	Porcine cartilage	0.48
Present study	0.58±0.20	22	0.70±0.24	22	Chick embryo cartilage	1.20

S.E.M.

observed diabetic mothers after an average gestation of 36.7 weeks were analysed for SM on the first and second day of life. These infants were compared with 9 others, average weight 2787 g, born to non-diabetic mothers after 37.4 weeks of gestation (Fig. 5). There was no significant difference between the mean values of SM in the two groups, nor from the mean value recorded on the second day of life in a group of normal newborns in the consecutive study described above.

SM in amniotic fluid. Ten tests were performed by amniocentesis at different times during gestation. The activity in all of the tests was found to be low (below 0.1 U/ml). Inhibitory activity, however, was observed in many of the tests.

DISCUSSION

The purpose of the present investigation was to assess the possible significance of maternal SM in fetal growth and production of SM in the newborn infant.

Although our results indicate that the SM activity was normal during the last trimester, it was lower at term than in normal non-pregnant women. Normal SM activity at term was reported by Chesley (2) who also found lower values in diabetic women during pregnancy

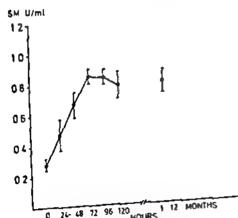


Fig. 4. Somatomedin in normal newborns in the first five days of life and the mean value in ten normal infants aged 1–12 months (mean ± S.E.M.).

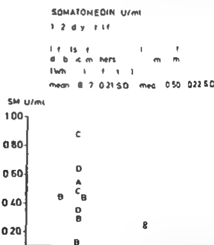


Fig. 5. Somatomedin in infants of diabetic mothers (classified according to White) compared to infants of normal mothers.

Likewise Daughaday (4) found normal SM in pregnancy at different stages

Somatomedin in mothers and newborns has been studied by several authors The results are summarized in Table 3 and compared with the results of the present investigation

Undoubtedly methodological conditions are partly responsible for the divergence in the results as well as the possible inhibitory influence of the estrogen and glucocorticoid content in serum on the bioassay method employed in the present investigation Other factors may also be important for example the concentration of the specific carrier protein as mentioned by D Ercole et al (5)

Since a positive correlation between maternal SM and the infant s length and weight was found in the present work this serves as evidence of a direct relationship between maternal SM and fetal growth A positive correlation between maternal SM and umbilical cord SM was also observed in the present investigation but this does not explicitly establish the presence of transplacental transport of SM The results do not exclude such a pathway but the relationship between maternal SM and fetal growth and endogenous SM production may be indirect in nature

Even though we were not able to demonstrate a positive relationship between maternal SM and placental weight in the present work this does not disallow the supposition of a stimulating effect of maternal SM on the placenta and through it upon fetal growth and endogenous SM production

According to this supposition the placental membrane contains a large number of SM receptors and D Ercole et al (5 6) proposed that in the sow the binding affinity on the maternal side is uniform throughout the gestation period whereas the specific binding sites affinity for SM increases during gestation in the fetal placental membrane In such a system an unchanged SM in the mother would cause an increased SM effect on the fetal side of the placenta

When evaluating this concept one should keep in mind the positive correlation found in this work between placental weight and birth weight and fetal SM activity HCS might also play an important role in such a system

Although it was not possible to demonstrate a correlation between HCS and SM or between HCS and birth weight this failure may be due to the time of sample collection Such a correlation might possibly be detected by testing 1–2 weeks before term as suggested by Gennazani et al (7) In a study which examined the relationship HCS/g placental tissue MacMillan (18) demonstrated a good correlation to weight and length

The above mentioned observations presumably support a concept of endogenous fetal production of SM during gestation the regulation and importance of which is unclear

The demonstration of a positive correlation between cord blood and birth weight suggests a role for SM in the control of fetal growth Similar findings have recently been reported by Gluckman et al (9) who also found positive correlation with birth weight length and head circumference and a rise in SM levels with gestational age

In the consecutive analyses carried out in the present work it was found that the SM activity rose during the course of the first week of life to the normal value

The rapid change in SM production probably is related to the sharp rise in GH secretion in that time period (8 14 16 21) but whether feedback regulation is present remains an open question

The detection of normal SM in the offspring of diabetic mothers would appear to exclude a relationship between SM and the higher birth weights observed in those children

By the radioreceptor assay high SM activity was measured in amniotic fluid (3 12 13 19) but whether this reflects fetal serum levels or has any relation to fetal growth has not yet been clarified

Although we were not able to discover a causal relationship between SM and fetal growth we believe that the results of this work

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Hintz et al (14)	0 50±0 19	52	0 41±0 20	28	Porcine cartilage	0 82
Swan et al (20)	0 55±0 05	12	0 70±0 08	12	Radioreceptor	1 27
Tato et al (21)	0 54±0 33	14	0 26±0 19	14	Porcine cartilage	0 48
Present study	0 58±0 20	22	0 70±0 24	22	Chick embryo cartilage	1 20

S E M

observed diabetic mothers after an average gestation of 36 7 weeks were analysed for SM on the first and second day of life These infants were compared with 9 others average weight 2 787 g born to non diabetic mothers after 37 4 weeks of gestation (Fig 5) There was no significant difference between the mean values of SM in the two groups nor from the mean value recorded on the second day of life in a group of normal newborns in the consecutive study described above

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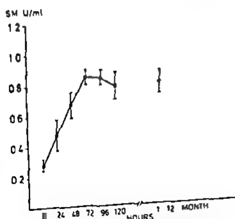


Fig 4 Somatomedin in normal newborns in the first five days of life and the mean value in ten normal infants aged 1–12 months (mean ± S E M)

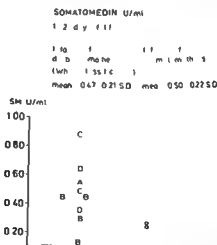


Fig 5 Somatomedin in infants of diabetic mothers (classified according to White) compared to infants of normal mothers

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PUBERTAL DEVELOPMENT IN THE PRADER LABHART WILLI SYNDROME

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From the Institute of Paediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva and the Sackler School of Medicine Tel Aviv University Israel

ABSTRACT Kauli R Prager Lewin R and Laron Z (Institute of Paediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva and the Sackler School of Medicine Tel Aviv University Israel) Pubertal Development in the Prader Labhart Willi syndrome Acta Paediatr Scand 67 763 1978 —The sexual maturation in the Prader Labhart Willi (PLW) syndrome was investigated in 14 patients 10 females and 4 males A wide variability in the pattern of pubertal development was found including delayed puberty in 5 patients and normal puberty in 4 patients sexual precocity was also observed in 5 patients true precocious puberty in one patient and incomplete sexual precocity in the form of precocious pubarche in 4 patients In 5 patients 3 of them with precocious pubarche the appearance of the pubertal signs was followed by a delay or arrest in their future development An LH RH stimulation test was performed in 11 patients In the 6 patients who eventually developed normal puberty the basal levels and the peak responses of both LH and FSH were within the range of those observed in normal controls of the same pubertal stage In 4 patients showing marked delay or arrest of puberty the basal levels were normal or low and the responses of LH and FSH to LH RH were blunted Priming with repeated LH RH stimulation in one of the male patients led to an augmented LH response suggesting a hypothalamic hypogonadotrophism It is concluded that the lack of uniformity in the pattern of sexual maturation in the PLW syndrome is due to a variability in the location and extent of a hypothalamic lesion which may comprise an active process continuing beyond the perinatal period

KEY WORDS Prader Labhart Willi syndrome normal puberty precocious puberty delayed puberty precocious pubarche

The Prader Labhart Willi (PLW) syndrome is considered to be a manifestation of hypothalamic dysfunction with a fairly consistent clinical expression Its diagnosis is based on the following clinical features a history of hypotonia in the neonatal period obesity from an early age mental retardation of varying degree short stature acromicria cryptorchidism and hypogonadism In the numerous publications on this syndrome in the literature which have appeared since its first description in 1956 (11) the process of sexual maturation has been reported to be usually delayed (5 6 8 9 11 14–17)

During the past 10 years we have followed in our Institute a total of 15 patients with the PLW syndrome One female patient died of a respiratory disease at the age of 5 years The remaining 14 patients were followed at regular intervals in our clinic The present report summarizes the dynamics of their pubertal development including studies of the hypothalamo-pituitary axis

SUBJECTS AND METHODS

The data on the pubertal development of these 14 patients with PLW syndrome are presented in Table I In all the diagnosis was based on the typical signs as originally described by Prader Labhart and Willi all have been markedly obese from an early age are mentally retarded and have a short stature acromicria and a typical facies with a fish like mouth In all anamnestic data provided evidence of hypotonia in the neonatal

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Age at last examination y m		Pubertal stage (1–5)	
CA	BA	Breast/ testes test vol	Pubic hair
1 ?	1 6	2	?–3
1? 4	1 6	3	3
13 ?	1 0	3–4	3
4 0	18 0	5 (0 cc)	5
15 0	14 0	4	4
16 9		4–5	5
0 8	16 0	?	3
4 0		1 (1 5 cc)	?
-5 0	19 0	1 (1 5 cc)	3
10 8	1? 0	2	2
1? 0	14 6	1	–3
15 0	14 6	4–5	5
18 0	18 0	3 (6 cc)	–3
1 0	19 0	5	5

at the age of 9½ years and incomplete sexual precocity in the form of precocious pubarche (adrenarche) in 3 females and one male On follow up it was observed that in 3 of the latter (2 females and 1 male) the precocious sexual hair failed to develop further with subsequent pubertal development being delayed or even permanently arrested The male patient from this group (AK) reached a testicular volume of 6 ml at the age of 16 years which was arrested at that size

3) Delayed appearance of the pubertal signs in 5 patients 3 females and 2 males both of whom also had cryptorchidism In 2 of the females puberty eventually developed normally with normal mensis but in the 2 male patients pubertal development was arrested after the age of 13 (MSh) and 14 (AG) years and their undescended testicles remained of a prepubertal size of 1 5 ml

Secretion of gonadotrophins

The individual values of the basal levels and peak responses to LH RH stimulation of plasma LH and FSH of 11 patients with the PLW syndrome who were subjected to an LH RH stimulation test are presented in Table 2 Two patterns of secretion were seen 1) In those patients who developed an active and progressive pubertal process the basal levels and peak responses of LH and FSH to LH RH stimulation were mostly within the normal range for their pubertal stage In the 3 female patients who had attained normal puberty (ShL ZE and AD) who were tested at mid cycle the peak responses for LH were exaggerated 2) In those patients in whom development of pubertal signs was subsequently arrested (TG AK HSh AG) even if they had shown precocious pubarche (TG and AK) the pattern of secretion of gonadotrophins was typical for hypogonadotrophic hypogonadism i e the basal levels were low or within normal limits and the response to LH RH stimulation was blunted One of these patients (AG) who was retested after priming with repeated stimulation with LH RH (3) showed a significant augmentation of the LH response but no change in the FSH response (Fig 1)

DISCUSSION

A survey of 14 patients with the PLW syndrome failed to reveal a uniform pattern of sexual maturation in contrast to numerous publications which report delayed puberty as the usual condition in these patients (5 6 9 11 15–17) In 9 of our 14 patients the appearance of pubertal signs was either normal or precocious In 4 patients incomplete sexual precocity was manifested as precocious pubarche (adrenarche) a finding which has previously been observed in this syndrome (J Neeman personal communication) There were no cases of precocious thelarche among the female patients and to the best of our knowledge such a manifestation has not yet been reported in this syndrome It is of note

Table 1 *Pubertal development in 14 patients with PLW syndrome*
CA=chronological age BA=bone age

Pattern of puberty	Name	Sex	Age at appearance of pubertal signs: Enlargement of breast/testes CA	BA	Pubic hair CA	BA	Evolution of puberty	Age at menarche or 1st ejaculation y m CA	BA
Normal puberty	HZ	F	11.0	11.6	10.6	11.0	Slow		
Normal puberty	AV	F	10.0		9.6		Normal		
Normal puberty	TL	F	10.0	8.4	10.0	8.4	Normal		
Normal puberty	NHO	M	12.0		12.0		Normal	15.0	
Delayed puberty	ShL	F	13.0	11.0	12.0	10.6	Slow eventually normal	14.0	11.0
Delayed puberty	ZE	F	14.0	13.0	13.0	11.0	Slow eventually normal	14.9	13.6
Delayed puberty	ZD	F	16.0	15.0	12.0	13.0	Slow		
Delayed puberty	HSh	M	Bilat cryptorchidism				Arrested at 13 yrs		
	AG	M	Bilat cryptorchidism		14.0		Arrested at 14 yrs		
Prec pubarche	RM	F	10.0	11.6	7.0	7.3	Slow		
Prec pubarche	TG	F			7.0	9.6	Arrested at 10 yrs		
Prec pubarche	AD	F	12.6	11.0	5.0		Slow eventually normal	14.0	13.0
Prec pubarche	AK	M	15.0	16.0	8.0		Arrested at age 14		
True prec puberty	FD	F	7.0		7.6		Fast	9.6	

Pubertal staging according to Tanner for breast/genitals and pubic hair (13)

period—in 8 there was a definite history and in 6 a suggestive history of weakness and unsteady head

The duration of follow up in these patients ranges from 2 to 12 years All patients have undergone a thorough clinical examination and evaluation of the signs of puberty 2 to 4 times a year Bone age was estimated once or twice a year on the basis of an X ray of the wrist in accordance with the Atlas of Greulich & Pyle (2) Pubertal staging was based on the criteria of Tanner for pubic hair genitalia and breast size (13) Testicular volume was estimated with an orchidometer (12 18) and penis length and width were measured with a simple caliper (4)

An LH RH stimulation test using a one bolus intravenous injection of 50 μg/m² of synthetic LH RH was performed in 11 patients 4 males and 7 females In the remaining 3 patients the test was not performed because of lack of cooperation on the part of the patients This test as well as the radioimmunoassay of the plasma LH and FSH were carried out as described previously (1 7) using as standards LER 960 for LH and LER 1344 for FSH The anti hLH serum was batch No 1 NPA and the anti hFSH serum was batch No 3 NPA Results are expressed as mIU of LH and FSH per ml plasma LER 907 serving as reference preparation The biological potency of 1 mg is 20 IU of FSH and 48 IU of LH One patient was retested after repeated stimulations with 100 μg LH RH i m daily for 5 days prior to retesting (3)

RESULTS

Clinical observations

As can be seen from Table 1 there was considerable variability in the time of onset of pubertal signs in this group of patients with three main patterns of pubertal development distinguishable

1) Puberty within normal limits as judged by the appearance of the pubertal signs at an expected age was seen in 4 patients 3 females and one male In one female patient from this group (HZ) further pubertal development was markedly slow but noticeable The remaining three patients of the group revealed a normal evolution of the pubertal process The only male patient with normal puberty (NHO) reached an adult testicular volume of 10 ml and had normal sexual activity

2) Sexual precocity was observed in 5 patients There was full true precocious puberty in one female patient who had her menarche

Age at last examination y m		Pubertal stage (1–5)	
CA	BA	Breast/ testes test vol	Pubic hair
	1 6		–3
4	1? 6	3	3
3?	1 0	3–4	3
4 0	18 0	5 (?0 cc)	5
15 0	14 0	4	4
6 9		4–5	5
0 8	16 0	?	3
4 0		1 (1 5 cc)	3
5 0	19 0	1 (1 5 cc)	3
10 8	1? 0	?	
1 0	14 6	1	2–3
15 0	14 6	4–5	5
18 0	18 0	3 (6 cc)	2–3
1 0	19 0	5	5

at the age of 9½ years and incomplete sexual precocity in the form of precocious pubarche (adrenarche) in 3 females and one male On follow up it was observed that in 3 of the latter (2 females and 1 male) the precocious sexual hair failed to develop further with subsequent pubertal development being delayed or even permanently arrested The male pa tient from this group (AK) reached a testicular volume of 6 ml at the age of 16 years which was arrested at that size

3) Delayed appearance of the pubertal signs in 5 patients 3 females and 2 males both of whom also had cryptorchidism In 2 of the females puberty eventually developed normally with normal mensis but in the 2 male patients pubertal development was arrested after the age of 13 (MSh) and 14 (AG) years and their undescended testicles remained of a prepubertal size of 1 5 ml

Secretion of gonadotrophins

The individual values of the basal levels and peak responses to LH RH stimulation of plasma LH and FSH of 11 patients with the PLW syndrome who were subjected to an LH RH stimulation test are presented in Table 2 Two patterns of secretion were seen 1) In those patients who developed an active and progressive pubertal process the basal levels and peak responses of LH and FSH to LH RH stimulation were mostly within the normal range for their pubertal stage In the 3 female patients who had attained normal puberty (ShL ZE and AD) who were tested at mid cycle the peak responses for LH were ex aggerated 2) In those patients in whom development of pubertal signs was subsequently arrested (TG AK HSh AG) even if they had shown precocious pubarche (TG and AK) the pattern of secretion of gonadotrophins was typical for hypogonadotrophic hypogonadism i e the basal levels were low or within normal limits and the response to LH RH stimulation was blunted One of these patients (AG) who was retested after priming with repeated stimulation with LH RH (3) showed a significant augmentation of the LH response but no change in the FSH response (Fig 1)

DISCUSSION

A survey of 14 patients with the PLW syndrome failed to reveal a uniform pattern of sexual maturation in contrast to numerous publications which report delayed puberty as the usual condition in these patients (5 6 9 11 15–17) In 9 of our 14 patients the appearance of pubertal signs was either normal or precocious In 4 patients incomplete sexual precocity was manifested as precocious pubarche (adrenarche) a finding which has previously been observed in this syndrome (J Neeman personal communication) There were no cases of precocious thelarche among the female patients and to the best of our knowledge such a manifestation has not yet been reported in this syndrome It is of note

Table 1 *Pubertal development in 14 patients with PLW syndrome*
CA=chronological age BA=bone age

Pattern of puberty	Name	Sex	Age at appearance of pubertal signs: Enlargement of breast/testes CA	BA	Pubic hair CA	BA	Evolution of puberty	Age at menarche or 1st ejaculation yrs CA	BA
Normal puberty	HZ	F	11 0	11 6	10 6	11 0	Slow		
Normal puberty	AV	F	10 0		9 6		Normal		
Normal puberty	TL	F	10 0	8 4	10 0	8 4	Normal		
Normal puberty	NHO	M	12 0		12 0		Normal	15 0	
Delayed puberty	ShL	F	13 0	11 0	12 0	10 6	Slow eventually normal	14 0	11 0
Delayed puberty	ZF	F	14 0	13 0	13 0	11 0	Slow eventually normal	14 9	13 6
Delayed puberty	ZD	F	16 0	15 0	17 0	13 0	Slow		
Delayed puberty	HSh	M	Bilat cryptorchidism				Arrested at 13 yrs		
	AG	M	Bilat cryptorchidism		14 0		Arrested at 14 yrs		
Prec pubarche	RM	F	10 0	11 6	7 0	7 3	Slow		
Prec pubarche	TG	F			7 0	9 6	Arrested at 10 yrs		
Prec pubarche	AD	F	12 6	11 0	5 0		Slow eventually normal	14 0	13 0
Prec pubarche	AK	M	15 0	16 0	8 0		Arrested at age 14		
True prec puberty	FD	F	7 0		7 6		Fast	9 6	

[a] Pubertal staging according to Tanner for breast/genitals and pubic hair (13)

period—in 8 there was a definite history and in 6 a suggestive history of weakness and unsteady head

The duration of follow up in these patients ranges from 2 to 12 years All patients have undergone a thorough clinical examination and evaluation of the signs of puberty 2 to 4 times a year Bone age was estimated once or twice a year on the basis of an X ray of the wrist in accordance with the Atlas of Greulich & Pyle (2) Pubertal staging was based on the criteria of Tanner for pubic hair genitalia and breast size (13) Testicular volume was estimated with an orchidometer (17 18) and penis length and width were measured with a simple caliper (4)

An LH RH stimulation test using a one bolus intravenous injection of 50 μg/m² of synthetic LH RH was performed in 11 patients 4 males and 7 females In the remaining 3 patients the test was not performed because of lack of cooperation on the part of the patients This test as well as the radioimmunoassay of the plasma LH and FSH were carried out as described previously (1 7) using as standards LER 960 for LH and LER 1344 for FSH The anti hLH serum was batch No 1 NPA and the anti hFSH serum was batch No 3 NPA Results are expressed as mIU of LH and FSH per ml plasma LER 907 serving as reference preparation The biological potency of 1 mg is 20 IU of FSH and 48 IU of LH One patient was retested after repeated stimulations with 100 μg LH RH i m daily for 5 days prior to retesting (3)

RESULTS

Clinical observations

As can be seen from Table 1 there was considerable variability in the time of onset of pubertal signs in this group of patients with three main patterns of pubertal development distinguishable

1) Puberty within normal limits as judged by the appearance of the pubertal signs at an expected age was seen in 4 patients 3 females and one male In one female patient from this group (HZ) further pubertal development was markedly slow but noticeable The remaining three patients of the group revealed a normal evolution of the pubertal process The only male patient with normal puberty (NHO) reached an adult testicular volume of 10 ml and had normal sexual activity

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…A	BA	Breast/ testes test vol	Pubic hair
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4	1? 6	3	3
?	1? 0	3–4	3
0	18 0	5 (0 cc)	5
0	14 0	4	4
9		4–5	5
8	16 0	?	3
0		1 (1 5 cc)	3
0	19 0	1 (1 5 cc)	3
–8	1 0		?
0	14 6	1	–3
0	14 6	4–5	5
0	18 0	3 (? cc)	–3
0	19 0	5	5

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Table 2. *Basal levels and peak values after LH-RH stimulation (50 µg/m² i.v.) of plasma LH and FSH in 11 patients with PLW syndrome*

CA=chronological age. BA=bone age. Pubertal staging according to Tanner for breasts/genitalia and pubic hair (13). In parenthesis—normal ranges for pubertal stages (Tanner for pubic hair)—from Dickerman et al. (1)

Name	Sex	Age at test, y.m.		Pubertal stage (1–5)		LH mIU/ml		FSH mIU/ml	
		CA	BA	Breast/testes	Pubic hair	Basal	Peak	Basal	Peak
HZ	F	11.3	11.6	1	2	0.5 (0.4–1.35)	2.61 (3.1–13.5)	0.87 (0.7–3.6)	5.76 (1.5–13.0)
ONH		24.0	18.0	5	5	0.79 (0.5–4.0)	15.32 (7.6–23)	2.99 (1.0–3.9)	6.37 (1.6–8.6)
ShL	F	14.0	11.0	2	3	1.2 (0.5–1.3)	20.2 (4.5–12.5)	0.55 (0.8–2.4)	10.48 (2.2–7.1)
ZI	F	15.6	14.0	3	3	1.88 (0.5–1.3)	23.7 (4.5–12.5)	5.54 (0.8–2.4)	9.04 (2.2–7.1)
ZD	F	17.0	15.0	2	2	0.4 (0.4–1.35)	6.0 (3.1–13.5)	1.88 (0.7–13.6)	7.58 (1.5–13.0)
HSh	M	24.0		1	3	0.54 (0.5–1.2)	0.65 (5.4–7.8)	0.5 (1.0–2.6)	5.76 (1.2–5.1)
AG	M	25.0	19.0	1	3	0.59 (0.5–1.2)	1.2 (5.4–7.8)	1.22 (1.0–2.6)	1.97 (1.2–5.1)
RM	F	10.6	12.0	1	2	0.28 (0.5–1.3)	0.95 (4.5–12.5)	0.94 (0.8–2.4)	2.1 (2.2–7.1)
TG	F	12.0	14.6	1	3	0.4 (0.5–1.3)	0.5 (4.5–12.5)	0.5 (0.8–2.4)	0.67 (2.2–7.1)
AD	F	13.5	12.0	3	4	1.84 (0.6–2.6)	24.85 (7.1–25.0)	3.17 (1.2–2.8)	7.07 (2.6–8.0)
AK	M	16.7	17.0	2	3	0.46 (0.5–1.2)	0.8 (5.4–7.8)	1.14 (1.0–2.6)	2.23 (1.2–5.1)

that in 3 of the 4 patients with precocious pubarche, sexual hair failed to develop beyond a certain stage and that these same patients later showed a delay or arrest of the pubertal process. Under normal conditions the appearance of sexual hair is largely determined by the adrenal androgens and it is possible that in these 4 patients the precocious pubarche was entirely or partly due to a precocious activation of the hypothalamo-pituitary-adrenal axis, in contradistinction to the lack of activation of the gonadotrophins. The hypogonadotrophism associated with the PLW syndrome has been suggested to be due to a hypothalamic dysfunction which in several instances was reported to have been overcome by prolonged administration of clomiphene (5, 9). While this drug was not given to any of our patients, the augmented response of LH seen in one patient (AG) following prolonged stimulation with LH-RH provides evidence for the existence of a hypothalamic hypogonadotrophism.

The variability in the pattern of puberty in the PLW syndrome can be explained by differences in the location and extent of the hypothalamic lesion. Furthermore, the subsequent delay or even arrest of the pubertal signs sometime after their appearance, as was seen in several of these patients, may denote a

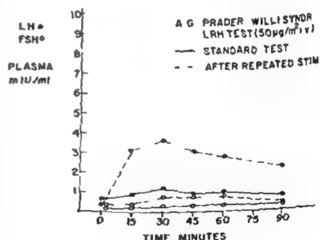


Fig. 1. LH-RH tests (50 µg/m² i.v.) in a male patient with PLW syndrome (AG at age 25)

tivity of the pathological process in the hypo thalamus beyond the perinatal period

Some investigators have raised the possibility that the defect in the PLW syndrome is not confined to the hypothalamo pituitary axis (10) Furthermore the possible coexistence of a primary hypogonadism in the PLW syndrome has been suggested in view of the absence of spermatogonia in a number of testicular biopsies made in such patients (6) In our series of patients the tendency toward a protracted pubertal development despite a normal or even precocious onset in the presence of a normal secretion of gonadotrophins (RM and ZD) and the exaggerated LH response to LH RH stimulation in some of the female patients (AD ShL and ZE) are also suggestive of a primary gonadal dysfunction

In conclusion it is clear from the findings of our investigation of 14 patients with the PLW syndrome that sexual development in this syndrome may follow different patterns To further clarify the nature and variability of the hypothalamic lesion in this condition it is necessary to carry out additional studies of the hypothalamo pituitary gonadal axis starting in prepuberty in individual patients

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		Age at test y m		Pubertal stage (1–5)		LH mIU/ml		FSH mIU/ml	
Name	Sex	CA	BA	Breast/ testes	Pubic hair	Basal	Peak	Basal	Peak
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ONH		24 0	18 0	5	5	0 79 (0 5–4 0)	15 32 (7 6–23)	2 99 (1 0–3 9)	6 37 (1 6–8 6)
ShI	F	14 0	11 0	2	3	1 2 (0 5–1 3)	20 2 (4 5–12 5)	0 55 (0 8–2 4)	10 48 (2 2–7 1)
ZE	F	15 6	14 0	3	3	1 88 (0 5–1 3)	23 7 (4 5–12 5)	5 54 (0 8–2 4)	9 04 (2 2–7 1)
ZD	F	17 0	15 0	2	2	0 4 (0 4–1 35)	6 0 (3 1–13 5)	1 88 (0 7–13 6)	7 58 (1 5–13 0)
HSh	M	24 0		1	3	0 54 (0 5–1 2)	0 65 (5 4–7 8)	0 5 (1 0–2 6)	5 76 (1 2–5 1)
AG	M	25 0	19 0	1	3	0 59 (0 5–1 2)	1 2 (5 4–7 8)	1 22 (1 0–2 6)	1 97 (1 2–5 1)
RM	F	10 6	12 0	1	2	0 29 (0 5–1 3)	0 95 (4 5–12 5)	0 94 (0 8–2 4)	2 1 (2 2–7 1)
TG	F	12 0	14 6	1	3	0 4 (0 5–1 3)	0 5 (4 5–12 5)	0 5 (0 8–2 4)	0 67 (2 2–7 1)
AD	F	13 5	12 0	3	4	1 84 (0 6–2 6)	24 85 (7 1–25 0)	3 17 (1 2–2 8)	7 07 (2 6–8 0)
AK	M	16 7	17 0	2	3	0 46 (0 5–1 2)	0 8 (5 4–7 8)	1 14 (1 0–2 6)	2 23 (1 2–5 1)

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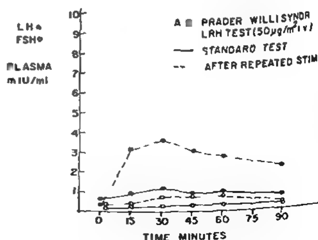


Fig 1 LH RH tests (50 µg/m² i v) in a male patient with PLW syndrome (AG at age 25)

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LONGTERM FOLLOW UP OF NEONATAL SFPTICEMIA

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ABSTRACT Alfven G Bergqvist G Bolme P and Eriksson M (Department of Paediatrics Karolinska Institute St Goran s Hospital Stockholm Sweden) Longterm follow up of neonatal septicemia Acta Paediatr Scand 67 769 1978.—The longterm prognosis of neonatal septicemia during the first four weeks of life has been estimated Of 90 infants with the diagnosis of neonatal septicemia during a five year period 1969–1973 65 infants survi ed the initial treatment Another two infants died with complications of their main disease intestinal atresia at the age of two months Thus the total mortality in neonatal septicemia in this series was 30% The remaining 63 children have been investigated between ages of 21/2 and 61/2 years Of these 63 children we have found 14 children (22% of the surviving) with handicaps where the septicemia can be regarded as a possible cause of the handicap Of these 14 children only six had an uncomplicated septicemia while four of them had meningitis and four had osteomyelitis Furthermore of the 14 handicapped children nine were delivered preterm (28–36 weeks) and all of them had one or more additional neonatal diagnoses than septicemia The prognosis both immediate and longterm of neonatal septicemia in the present series compares favourably to most international studies The importance of early detection together with an aggressive treatment of the septicemia is stressed and is considered as the main reason for the good prognosis

Several reviews have been written to date concerning neonatal septicemia (1 2 3 7 8 20 23) These reviews deal primarily with predisposing and etiological factors while follow up studies are very rare A few reports on the longterm prognosis following neonatal meningitis and septicemia are however available (5 6 10 14 18 19 20) They all include few patients (20–25 children) and agree on a high mortality rate as well as a significant number of children with permanent sequelae It has also been suggested that an improvement in the survival rate would be at the expense of major neurological sequelae in the survivors (14)

In addition there have been studies on another complication to neonatal septicemia osteomyelitis—osteoarthritis (15 22) Those studies point to a higher incidence of permanently impaired function of the affected limb in infants than in older children

In order to study the longterm prognosis in an unselected material we have examined 63 children surviving from neonatal septicemia

MATERIAL

Data were collected on 90 children with the diagnosis of neonatal septicemia below the age of four weeks treated at the Department of Pediatric and Pediatric Surgery at St Goran s Hospital during the years 1969–1973 During this period the initial antibiotic treatment in most cases of the infants consisted of kanamycin ampicillin and cloxacillin for 8–12 days Included in the survey are both infants with primary septicemia and with secondary septicemia i e septicemia in children who had prior to the diagnosis been subject to some kind of surgery Eleven infants referred from other hospitals for intensive c re treatment with the diagnosis of neonatal septicemia are also included Of the 90 infants 25 died within four weeks after the diagnosis (Table 1) The present report concerns the initially surviving 65 infants Most of the children have been followed during the first year at the outpatient clinic as part of the routine follow up of high risk infants All children could then be traced through their child health centers Three children had moved abroad and information was obtained from their relatives and examinations at the child health center before they moved at or 4 years of age Eleven children had moved from the Stockholm area and they were examined by pediatricians at their

Table 3 *Detailed survey of children with handicaps following neonatal septicemia*

SGA=small for gestational age CP=cerebral palsy IRDS=idiopathic respiratory distress syndrome p-m=psychomotor MBD=minimal brain damage TGA=transposition of great arteries

Case no	Age at the investigation (years)	Neonatal diagnosis	Outcome
1	6½	Preterm 34 weeks E coli meningitis	Hydrocephalus with shunt
	6½	Preterm 33 weeks IRDS hyperbilirubinemia Staph aureus septicemia osteomyelitis	Late p m development pes equinovarus
3	6½	Preterm 8 weeks IRDS respirator treatment 1 year pseudomonas septicemia	MBD slight hearing defect late p-m development
4	5	Maternal diabetes hypoglycemia fetal asphyxia (Apgar ? min 4) Staph aureus osteomyelitis	Shorter arm (1 cm)
5	5	Hyperbilirubinemia β Streptococcus group B septicemia	MBD late p-m development pes equinovarus
[illegible]	5	Fetal asphyxia (Apgar 1 min 2 5 min 6) Klebsiella septicemia	Late p m development
7	4½	Preterm 32 weeks IRDS hyperbilirubinemia Klebsiella meningitis	Slight hearing defect
8	4½	Preterm 33 weeks hyperbilirubinemia Klebsiella meningitis	Moderate hearing defect
9	4½	Twin SGA Staph aureus meningitis	Hydrocephalus with shunt
10	4	TGA Staph aureus osteomyelitis	Shorter leg (1 cm)
11	4	Preterm 9 weeks hyperbilirubinemia haemorrhagia cerebri E coli septicemia	Op cerebral tumor (ependyom stage II) severe hearing defect
1	3	Twin preterm 35 weeks neonatal convulsion Staph albus septicemia	Blind severe CP severe mental retardation
13	3	Preterm 36 weeks IRDS hyperbilirubinemia Staph aureus septicemia osteomyelitis	Shorter leg (4 cm) walking with limp
14	?½	Preterm 30 weeks E coli septicemia	CP diplegia late p m development epilepsia

with severe VOC Of the handicapped children in Table 3 case 12 had neonatal convulsions for which we could find no etiology and it is not likely that the handicaps were caused by the septicemia Further in case 11 it was not clear if the child had her hearing defect before the operation for the cerebral tumor As can be seen in the whole group of children other risk factors than the septicemia were also common Hearing defects were observed in two of the children who also had had meningitis The two infants with Klebsiella meningitis were treated first with kanamycin then gentamycin Since the plasma concentrations of kanamycin and gentamycin were not routinely measured at the time of the septicemia in the present infants we cannot speculate if aminoglycoside toxicity could play any part in the hearing abnormalities

DISCUSSION

Several authors have stressed the poor prognosis both immediate and the longterm after neonatal septicemia (5 6 7 8 9 10 14 18 19 20) In this review we found an initial mortality rate of 28% and a total mortality of 30%

In neonatal meningitis mortality rates of 40–60% have been found (5 6 14 18 19 20) In our review we found a mortality rate of 35% mainly following gram negative rod infection In addition to a high mortality rate in the above referred studies the incidence of permanent sequelae was reported to be approximately 50% Of 125 survivors collected from 12 publications on neonatal meningitis and reported by Fosson & Fine 36% had severe neurologic impairments (6) Moreover in another study based on a series of 82 infants it

Table 1 *Causative organism and immediate complications in 90 infants with neonatal septicemia*

Isolated organism	Total	Dead within four weeks	Meningitis	Osteomyelitis
Staphylococcus	29	4	2	10
Streptococcus (group B)	12	0	3	0
Enterococcus	6	3	1	0
E Coli	24	12	9	0
Other gramnegative rods (Pseudomonas Klebsiella Enterobacter Coliform)	19	6	5	1
Total	90	25	20	11

Two infants operated on because of intestinal atresia died at two months of age

residence The remaining 50 children participated in a follow up examination at St Goran s Hospital by three of the authors The examination consisted of an interview concerning development and previous health and an examination with special attention to neurological hearing and speech abnormalities All records from child health centers and hospitals were studied Of the total of 63 surviving children 34 had been subject to the special check up that with small local modifications are offered to all four year old children in Sweden (13 21)

RESULTS

The immediate prognosis and the complications within four weeks after the diagnosis are shown in Table 1 and are related to the causative organism The mortality rate in the group of infants with meningitis was 35% (7/20) with the highest incidence with *E coli* meningitis 56% (5/9) Two children with gastrointestinal malformations died from surgical complications at the age of two months

At the time of the follow up investigation all surviving 63 children were living with their parents Six parents considered their children to have more infections than other children of the same age In only two instances however had these infections (pneumonias) led to hospital care At the follow up investigation or at their last examination at the child health center their length weight and head circumference were measured No trend of abnormality in any of these parameters was observed in relation to the standard for Swedish children (11) Even the infants who were small for gestational age at birth (six surviving out of eleven) now had normal weight and length according to age (within two standard deviations of the mean)

The median age for walking without support was 13 7 months with a range of 9–24 months Late walking ability was seen in two children with severe congenital heart disease (22 and 24 months) in one child with pes equinovarus (18 months) and in one child with pronounced difference in leg length following osteomyelitis (18 months) In two children a permanent squint was noted and in a further two it was suspected Convulsions were present in only one child with a spastic diplegia

The findings at the follow up examination are summarized in Table 2 The handicaps found are further presented in Table 3 Of the three children with handicaps considered not to be related to their septicemia were one child with Down s syndrome and two children with somewhat late motor development together

Table 2 *Findings at follow up examination of 63 surviving children with neonatal septicemia*

	No	%
Normal development	46	73
Handicap not related to the septicemia	3	
Handicap possibly related to the septicemia	14	22

For details of these children see Table 3

Table 3 *Detailed survey of children with handicaps following neonatal septicemia*
SGA=small for gestational age CP=cerebral palsy IRDS=idiopathic respiratory distress syndrome p-m=psychomotor MBD=minimal brain damage TGA=transposition of great arteries

Case no	Age at the investigation (years)	Neonatal diagnosis	Outcome
1	6½	Preterm 34 weeks E coli meningitis	Hydrocephalus with shunt
	6½	Preterm 33 weeks IRDS hyperbilirubinemia Staph aureus septicemia osteomyelitis	Late p-m development pes equinovarus
3	6½	Preterm 28 weeks IRDS respirator treatment 1 year pseudomonas septicemia	MBD slight hearing defect late p-m development
4	5	Maternal diabetes hypoglycemia fetal asphyxia (Apgar 2 min 4) Staph aureus osteomyelitis	Shorter arm (1 cm)
5	5	Hyperbilirubinemia β Streptococcus group B septicemia	MBD late p-m development pes equinovarus
6	5	Fetal asphyxia (Apgar 1 min ; 5 min 6) Klebsiella septicemia	Late p-m development
7	4½	Preterm 37 weeks IRDS hyperbilirubinemia Klebsiella meningitis	Slight hearing defect
8	4½	Preterm 33 weeks hyperbilirubinemia Klebsiella meningitis	Moderate hearing defect
9	4½	Twin SGA Staph aureus meningitis	Hydrocephalus with shunt
10	4	TGA Staph aureus osteomyelitis	Shorter leg (1 cm)
11	4	Preterm 29 weeks hyperbilirubinemia haemorrhagia cerebri E coli septicemia	Op cerebral tumor (ependyom stage II) severe hearing defect
12	3	Twin preterm 35 weeks neonatal convulsion Staph albus septicemia	Blind severe CP severe mental retardation
13	3	Preterm 36 weeks IRDS hyperbilirubinemia Staph aureus septicemia osteomyelitis	Shorter leg (4 cm) walking with limp
14	2½	Preterm 30 weeks E coli septicemia	CP diplegia late p-m development epilepsia

with severe VOC Of the handicapped children in Table 3 case 12 had neonatal convulsions for which we could find no etiology and it is not likely that the handicaps were caused by the septicemia Further in case 11 it was not clear if the child had her hearing defect before the operation for the cerebral tumor As can be seen in the whole group of children other risk factors than the septicemia were also common Hearing defects were observed *in two* of the children who also had had meningitis The two infants with Klebsiella meningitis were treated first with kanamycin then gentamycin Since the plasma concentrations of kanamycin and gentamycin were not routinely measured at the time of the septicemia in the present infants we cannot speculate if aminoglycoside toxicity could play any part in the hearing abnormalities

DISCUSSION

Several authors have stressed the poor prognosis both immediate and the longterm after neonatal septicemia (5 6 7 8 9 10 14 18 19 20) In this review we found an initial mortality rate of 28% and a total mortality of 30%

In neonatal meningitis mortality rates of 40–60% have been found (5 6 14 18 19 20) In our review we found a mortality rate of 35% mainly following gram negative rod infection In addition to a high mortality rate in the above referred studies the incidence of permanent sequelae was reported to be approximately 50% Of 125 survivors collected from 12 publications on neonatal meningitis and reported by Fosson & Fine 36% had severe neurologic impairments (6) Moreover in another study based on a series of 82 infants it

Table 1 *Causative organism and immediate complications in 90 infants with neonatal septicemia*

Isolated organism	Total	Dead within four weeks	Meningitis	Osteomyelitis
Staphylococcus	29	4	2	10
Streptococcus (group B)	12	0	3	0
Enterococcus	6	3	1	0
E Coli	24	12	9	0
Other gramnegative rods (Pseudomonas Klebsiella Enterobacter Coliform)	19	6	5	1
Total	90	25	20	11

Two infants operated on because of intestinal atresia died at two months of age

residence The remaining 50 children participated in a follow up examination at St Goran s Hospital by three of the authors The examination consisted of an interview concerning development and previous health and an examination with special attention to neurological hearing and speech abnormalities All records from child health centers and hospitals were studied Of the total of 63 surviving children 34 had been subject to the special check up that with small local modifications are offered to all four year old children in Sweden (13 21)

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was shown that 31% had evidence of impairment (hydrocephalus mental retardation and neurologic deficit) present at discharge (18) In the present survey we found that four of the 14 handicapped children had had meningitis These four children constitute 30% of the surviving infants with neonatal meningitis In three of the four cases the causative organism was a gram negative rod The less favourable prognosis in gram negative meningitis has been observed by other authors and has been attributed to a diminished rate of killing of the gram negative bacteriae in response to antimicrobial therapy (16)

The main problem with osteomyelitis in the neonatal period is the high rate of joint involvement (22) Functional deficit has been found in approximately 50% of infants with osteomyelitis (15 22) In our study four handicapped children had sequelae from staphylococcal osteomyelitis This constitutes more than one third of the infants with osteomyelitis Two of these infants had had indwelling umbilical artery catheters during treatment of IRDS and a third child had undergone cardiac catheterization to diagnose transposition of the great arteries The complications such as osteoarthritis following umbilical artery catheterization have received increasing attention in recent years (12)

An increased incidence of group B streptococcal infections in infants has been reported during the last decade (2 7) Thus Horn et al reported a combined morbidity and mortality rate of 50% (10) whereas all 12 infants in our study survived with a slight handicap in only one child

When discussing mortality and morbidity one has to remember that most of the children with neonatal septicemia have predisposing risk factors that also contribute to the later occurrence of handicaps (1 8) As can be seen in Table 3 all the handicapped children had at least one additional diagnosis Slight hearing loss may relate to the treatment with aminoglycosides (4 9 17) In newborns following treatment with streptomycin the combination of streptomycin treatment and other factors such as hyperbilirubinemia was found to lead to the hearing loss (9) Two of the four children in the present study with hearing loss had had both meningitis and hyperbilirubinemia in addition to the treatment with aminoglycosides

The rather favourable prognosis in the present study in comparison to other reports is encouraging We would suggest that this might be due to early detection and treatment as well as aggressive and intensive attention to supportive care as e g artificial respiration plasma expanders etc Thus early diagnosis as well as early treatment should be stressed as of utmost importance for the final outcome of neonatal septicemia

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TWO FORMS OF CUTIS LAXA PRESENTING IN THE NEWBORN PERIOD

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ABSTRACT Agha A Sakati N O Higginbottom M C Jones K L Jr Bay C and Nyhan W L (Departments of Pediatrics As-Salama Hospital and Ash-Sharq Hospital Saudi Arabia and the University of California San Diego La Jolla California USA) Two forms of cutis laxa presenting in the newborn period Acta Paediatr Scand 67 775 1978.—Two infants are described with congenital cutis laxa They represent two distinct disorders In the first congenital cutis laxa is associated with a generalized disorder of elastic tissue in which there may be diaphragmatic or other hernias diverticula of the gastrointestinal or urinary tract and infantile emphysema The disease is fatal often within the first year In the second congenital cutis laxa is associated with widely patent anterior fontanel a variety of malformations and retarded growth and development Recognition of these distinct syndromes in the newborn period and their recessive inheritance permit realistic discussion of the prognosis which is very different from the benign dominant forms of cutis laxa

KEY WORDS Cutis laxa emphysema diaphragmatic hernia retarded development delayed closure of the anterior fontanel

Cutis laxa is a descriptive term referring to skin that is loose but not hyperelastic It has been used to designate a variety of unrelated disorders Beighton (1) distinguished between two forms of cutis laxa a relatively benign autosomal dominant form frequently presenting first in adult life and an autosomal recessive form usually lethal in infancy

Experience with the two patients that make up this report indicates that there are at least two conditions that present with cutis laxa very early in life It is the purpose of this report to delineate these two distinct disorders

CASE REPORTS

Case 1 I W A a male was noted at birth to have an unusual old appearing face and loose inelastic skin Birth was at term to a 6-year-old Saudi Arabian mother Her first baby had died at the age of 10 days of unknown cause Two female siblings were well The parents were first cousins Pregnancy was complicated by hepatitis during the last week Labor and delivery were uneventful The birth weight was 2980 g The skin was loose and redundant and it assumed many folds (Fig 1) There was bilateral blepharochalasis The corners of the mouth drooped The mouth was wide and the palate high arched

The lungs were clear There was a grade III/VI precordial systolic murmur He had bilateral inguinal hernias A congenital dislocation of the right hip was confirmed roentgenographically (Fig 2)

The infant appeared generally well but he had some difficulty breathing during feeding Roentgenogram of the chest revealed a large diaphragmatic hernia (Fig 3) An intravenous pyelogram revealed a diverticulum of the bladder (Fig 4) The serum copper was 8 3 µmol/l (53 µg/100 ml) (normal 11–24 µmol/l) The level of ceruloplasmin was normal Biopsy of the skin showed a decrease in the number of elastic fibers with splaying and fragmentation of existing fibers consistent with a diagnosis of cutis laxa

Surgery was performed at 2 weeks of age The stomach and duodenum were brought down from the chest and the hernia repaired The patient did well postoperatively Two months later he developed diarrhea and a cough and was admitted to hospital Roentgenogram of the chest revealed a minimal infiltrate in the left upper lobe He was rehydrated and discharged after two weeks One month following discharge the baby was found dead in bed Autopsies are not done in Saudi Arabia

Case 2 A H was born at term in Mercy Hospital San Diego after an uncomplicated 42 weeks pregnancy



Fig 4 Case 1 Intravenous pyelogram illustrates a diverticulum of the bladder



Fig 5 Case 2 In this newborn infant with cutis laxa the thick folds of excess skin are evident over the trunk and extremities She had a large umbilical hernia

Developmental evaluation at 11 months of age using the Bayley Scales of Infant De elopment revealed a delay in gross motor development for which she scored at the 7 month level In contrast her mental development score was at the 9 month level At 4 months using the Vineland Social Maturity Scale she was felt to be functioning within the a erage range of intelligence but she had a 6 month delay in motor skills

DISCUSSION

These infants represent two of the disorders that present with cutis laxa in the newborn period cutis laxa with emphysema patient 1 and cutis laxa with retarded development patient 2 The first disorder usually leads to death within the first years of life from pulmonary complications The second disorder is not associated with pulmonary disease but there are many systemic defects among which gross delay in motor development is the most important

The principal features that differentiate these two disorders are set out in Table 1 An autosomal dominant form of cutis laxa may also be seen in the newborn period We have summarized the findings in 8 patients with cutis laxa with retarded development (7 from



Fig 1 Case 1 The redundant skin over the scalp is representative of the skin of the entire body

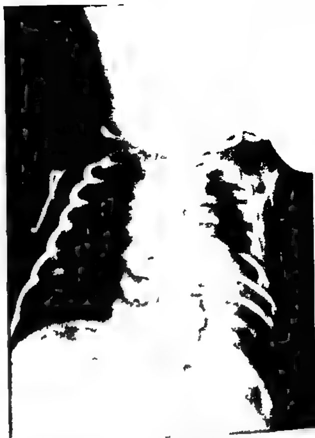


Fig 3 Case 1 The diaphragmatic hernia is well visualized with contrast medium in the upper gastrointestinal tract

labor and delivery to a 19 year old Black woman The father was 24 years old and there was no consanguinity A 2 year old half sister was normal

Physical examination at birth revealed a jittery baby with increased muscle tone and generalized cutis laxa The length was 49 cm (25th percentile) weight 2743 g (10th percentile) and the head circumference 31 cm (below the 3rd percentile) The anterior fontanel measured 6×6 cm There was an antimongoloid obliquity and slight nasal flattening The philtrum measured 1 cm The palate was high and V shaped She had a prominent reducible umbilical hernia The hips were dislocated bilaterally There were talipes equinovarus and severe metatarsus adductus bilaterally There was a simian crease on the left hand and hyperextensible fingers Marked laxity of the skin was present throughout the body and included the face and the eyelids (Fig 5)

The ceruloplasmin was 0 199 g/l which is normal The serum copper was 11 µmol/l (70 µg/100 ml) The T_4 was 88 nmol/l The total protein was 75 g/l the albumin was 48 and the globulins $\alpha 1$ 4 α^2 8 β 9 and γ 6 g/l

The feet were treated in plaster casts The hips were treated with a brace By 11 months of age she was sitting but with a rounded back and could say one word The fontanel was 5×5 cm The weight of 8 3 kg was in the 25th percentile A left hydronephrosis due to ureteropelvic junction obstruction was discovered by intravenous pyelogram and left antegrade pyelography A pyeloplasty was performed Microscopic sections showed normal ureteral and renopelvic junction mucosa and connective tissue Elastic stains revealed a fine elastic network consisting of fibers with the normal angular appearance Roentgenograms with contrast medium in the gastrointestinal tract failed to reveal any herniations or diverticuli

At 24 months her length was 88 cm (75th percentile) and head circumference was 46 5 cm (25th percentile) weight was 9 5 kg (less than 3rd percentile) The anterior fontanel measured 3×4 cm Her inner canthal distance was 2 8 cm (75th percentile) The skin remained generally lax The cheeks had begun to droop laterally as did the lower lip All of the joints were hyperextensible except for her hips which were difficult to abduct but roentgenographs of the hips were normal



Fig 2 Case 1 The hips were congenitally dysplastic

ly in cutis laxa with retarded development A large fontanel with delayed closure and ocular hypertelorism are particularly helpful in differentiating this disorder from the other two conditions (Table 1) Additional craniofacial abnormalities occasionally observed in this disorder include epicanthal folds bilateral macular colobomas fine retinal pigmentary changes a small mouth high arched palate and low set ears

Cutaneous manifestations are similar in all three disorders but they are much less severe in autosomal dominant cutis laxa In cutis laxa there are myriad folds of loose redundant skin about the trunk and extremities The face is prominently involved and the drooping mouth and lower lids give the patient an aged appearance even in infancy Ectropion and blepharochalasis of the lids are common The skin tends to sag in folds The genitalia may be entirely covered by folds of skin It is important to distinguish cutis laxa from the Ehlers Danlos syndrome The skin is hyperextensible but not hyperelastic—while in the Ehlers Danlos syndrome the skin is hyperelastic but not lax In cutis laxa when the skin is pulled out it only slowly reassumes its normal position It does not bruise or split easily and it heals normally

Potentially lethal systemic defects involving the respiratory system are seen only in cutis laxa with emphysema as illustrated by patient 1 The most serious complication is emphysema which may be present from birth Patients may have tachypnea prolonged expiration or apparent asthma Progressive pulmonary insufficiency (10) which may be documented by abnormal tests of pulmonary function frequently leads to cor pulmonale with electrocardiographic evidence of right ventricular hypertrophy and right bundle branch block These complications or pneumonia led to death prior to two years of age in three of the patients Affected individuals may develop diverticuli in the esophagus stomach duodenum and ileum (10) Diaphragmatic hernia may be an early complication (10) and inguinal and umbilical hernias are common (5 10) Prolapse of gastric rectal and vaginal mucosa have been documented There may also be diverticuli of the bladder (6) or elongated redundant ureters Frequent urination may be a symptom

Nonprogressive systemic defects such as simian creases high arched palate colobomas or ocular laxa may be seen in any of the syndromes of cutis laxa In each of these syndromes the voice is hoarse harsh or prematurely deep On laryngoscopy the vocal chords may appear elongated Congenital dislocation of one or both hips is a consistent feature of cutis laxa with retarded development but it was also present in our patient with cutis laxa with emphysema

In addition to the three conditions summarized in Table 1 DeBarsey et al (7) and subsequently Burck (4) have delineated a multiple malformation syndrome the principal features of which are generalized cutis laxa pre and post natal deficiency of growth severe mental retardation athetosis large fontanels hypertelorism and cloudy corneas The features which distinguish this disorder from the syndrome of cutis laxa with retarded development are cloudy corneas the severity of the mental deficiency and athetosis The patient reported by Kaye et al (13) may represent an additional distinct disorder In this patient cutis laxa was associated with retardation of intrauterine and extrauterine growth defective ossification of the parietal bones and ambiguous genitalia

In the literature the genetics of cutis laxa are not really clear Milder forms of cutis laxa most of which appear first in adolescence or adult life are dominantly inherited (1 2 18) Evidence of transmission from a male parent to a male child has been reported (1 19) and advanced maternal age has been documented in sporadic cases (2) We believe that the forms of cutis laxa reported here are recessive There have been at least two examples of parental consanguinity in each disorder and there have been a number of affected siblings

Table 1 *Clinical features of three forms of cutis laxa which present in the newborn period*

Anomaly		Cutis laxa with retarded development	Cutis laxa with emphysema	Dominant cutis laxa
Growth and performance	Prenatal growth deficiency	+	−	−
	Postnatal growth deficiency	+	+	−
	Developmental delay	+	−	−
Craniofacial	Large fontanels with delayed closure	+	−	−
	Ocular hypertelorism	+	−	−
Connective tissue	Cutis laxa	+	+	+
	Potentially lethal systemic defects	−	+	−
	Nonprogressive systemic defects	+	+	+
	Congenital hip dislocation	+	±	−
	Lax joints	+	−	−
Inheritance		Autosomal recessive	Autosomal recessive	Autosomal dominant

the literature) (3 8 9 17) 10 patients with cutis laxa with emphysema (9 from the literature) (1 5 6 10 11 15 19), and 10 patients with autosomal dominant cutis laxa (2 12 14 16 18) A number of other patients that have been reported cannot be classified from the data available as either dominant or recessive

Deficiency of prenatal growth has been seen only in cutis laxa with retarded development At birth the average length and weight of 7 patients was 45 cm and 2 1 kg both of which represented the 50th percentile for 34 weeks gestation Postnatal retardation of growth has been seen in both cutis laxa with emphysema and cutis laxa with retarded development In the former condition this is likely related to the serious often lethal systemic complications of the syndrome In cutis laxa with retarded development all of the 5 patients from the literature who had been followed past one year of age had heights in the 10th percentile or below for their chronologic ages and each was underweight for height In our patient there was a striking deficiency of growth in weight but postnatal deficiency of linear growth was not present At 24 months her length was in the 75th percentile while her weight was less than the 3rd percentile

Normal intellectual development has been reported in each of the 4 patients with cutis laxa with emphysema in whom data are available and in all of the patients with the autosomal dominant type of cutis laxa Developmental delay manifested primarily by gross motor retardation has been reported in all patients with cutis laxa with retarded development followed past the newborn period One of the patients reported by Reisner (17) was noted at 4 years of age to have slow development Now 10 years old she has an IQ of 50 The other patient her sister is now 8 years of age She is functioning normally in regular school despite failing the first grade Both patients followed past the newborn period in the report of Bittell Dobrzynska (3) had delayed motor development One had poor sucking behavior in the newborn period and at 13 months of age was unable to sit alone The second patient did not walk until three years However she was an average student in school at 11 years of age In our patient gross motor function was severely delayed At 24 months she was unable to pull herself up crawl or walk However she had an otherwise average intellectual performance

Craniofacial abnormalities occur consistent

ly in cutis laxa with retarded development A large fontanel with delayed closure and ocular hypertelorism are particularly helpful in differentiating this disorder from the other two conditions (Table 1) Additional craniofacial abnormalities occasionally observed in this disorder include epicanthal folds bilateral macular colobomas fine retinal pigmentary changes a small mouth high arched palate and low set ears

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of normal parents (10 17) All 8 patients reported with cutis laxa with retarded development have been females This suggests the possibility of X linked dominant inheritance in which the hemizygous condition in the male is lethal The number of patients is too small to assess the validity of this trend

The patients described by DeBarsey et al (7) and by Burck (4) were sporadic as was the patient reported by Kaye et al (13)

The major importance of the recognition of cutis laxa in the newborn period is its implication for prognosis The young infant with cutis laxa should be considered at risk for the development of serious life threatening pulmonary disease and a fever should be thought likely to imply pneumonia unless the child meets the criteria for one of the other disorders set out in Table 1 In cutis laxa with retarded development the presence of a widely patent fontanel and a variety of minor anomalies may suggest a guarded prognosis as to intellectual development

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OBSERVATIONS ON INTRAUTERINE GROWTH IN URBAN ETHIOPIA

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ABSTRACT Gebre Medhin M Sterky G and Taube A (Institute of Nutrition University of Uppsala Uppsala Department of Paediatrics Karolinska Institute St Goran s Hospital Stockholm and Department of Statistics University of Uppsala Uppsala Sweden) Observations on intrauterine growth in urban Ethiopia Acta Paediatr Scand 67 781 1978.—A total of 3144 deliveries comprising a wide range of socio-economic groups in Addis Abeba were investigated A positive correlation was found between family income and birth weight with a difference of nearly 500 g between the extremes of socio-economic classes A seasonal variation in mean birth weight was also observed The mean length of gestation was 7 4 days shorter and the anthropometric measures at birth were significantly lower in the Ethiopian infants as compared with the Swedish norm The median weight and length development in relation to gestational age in the Ethiopian newborns was similar to the Swedish standard up to approximately 34–35 weeks of gestation After that time there was very little further intrauterine growth in Ethiopian infants The possible reasons for the observed pattern of growth are discussed

KEY WORDS Intrauterine growth gestational age developing country socio-economics season malnutrition

n Ethiopia (11 41) as in many other developing countries the proportion of low birth weight (LBW) infants—those who weigh ≤2 500 g—is much higher than in affluent societies (39 40) The possibility that these infants are full term babies has raised the question whether their small size reflects intrauterine growth retardation (17)

In well developed areas the availability of intrauterine growth standards against which newborn infants can be evaluated has proved useful in the analysis of data for planning purposes and in the care of those who may have been born preterm or have grown slowly because of an adverse intrauterine environment (20 36) In less developed countries where perinatal mortality rates are still excessively high (40) such standards are mostly lacking and none exist for Ethiopia

The present investigation was conducted in order to elucidate the pattern of intrauterine growth in an urban Ethiopian community and to find out to what extent the high incidence of LBW described earlier (11 41) is due to retardation of fetal growth or to shortened gestational age

MATERIAL AND METHODS

Clinical material

This survey forms part of a comprehensive study of the nutritional aspects of pregnancy conducted at the Ethiopian Nutrition Institute (ENI) in Addis Abeba during the years 1969–75 The subjects were recruited from two maternity units the Lideta Mother and Child Health Training and Demonstration Center which caters for the indigent and the Ghandi Memorial Hospital to which paying mothers come These centers constitute two out of a dozen institutions in the capital which provide maternity care occasionally in combination with general curative services

Practically all singleton liveborn infants delivered at these two centers on Monday through Friday during the 1 month period January to December 1971 were included in the survey The reasons for the exclusions which amounted to about 6% of all the relevant deliveries in both

of normal parents (10 17) All 8 patients reported with cutis laxa with retarded development have been females This suggests the possibility of X linked dominant inheritance in which the hemizygous condition in the male is lethal The number of patients is too small to assess the validity of this trend

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In Ethiopia (11 41) as in many other developing countries the proportion of low birth weight (LBW) infants—those who weigh ≤2 500 g—is much higher than in affluent societies (39 40) The possibility that these infants are full term babies has raised the question whether their small size reflects intrauterine growth retardation (17)

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Table 1 *Birth weight means median and standard deviation by sex and gestational age*

Gestational age (weeks)	No of cases	Mean BW (g)	S D (g)	Median BW (g)	Smoothed median BW (g)
Male					
34	28	3 000	590	3 000	
35	45	3 090	490	3 200	3 130
36	81	3 120	560	3 200	3 170
37	158	3 090	480	3 100	3 170
38	383	3 160	430	3 200	3 200
39	493	3 230	440	3 300	3 270
40	239	3 230	460	3 300	3 300
41	97	3 240	430	3 300	3 300
42	65	3 280	450	3 300	3 300
43	76	3 240	430	3 300	
Female					
34	24	2 800	460	2 900	
35	47	3 110	470	3 100	3 000
36	80	2 980	390	3 000	3 000
37	118	2 940	460	2 900	2 970
38	343	3 050	360	3 000	3 000
39	445	3 170	390	3 100	3 100
40	211	3 150	430	3 200	3 130
41	72	3 170	490	3 100	3 170
42	40	3 160	460	3 200	3 200
43	61	3 290	410	3 300	

centers for the duration of the study were either Caesarean sections or specified disease in the mother or child (diabetes hypertension stillbirths congenital malformations) or uncertainty concerning the length of gestation None had to be excluded because of cigarette smoking as this is hardly ever encountered among Ethiopian women At both centers only a few of the women had any regular antenatal care

The mothers mainly belonged to the four closely inter related ethnic groups of highland Ethiopia and were from widely varying socio economic backgrounds

Examination methods

Staff The survey was performed by a team consisting of eight specially trained interviewers with a secondary school background one registered nurse and two physicians in addition to the ordinary staff of the maternity centers

Determination of gestational age The length of gestation was calculated from the first day of the last menstrual period by interviewing the mothers using a specially designed local calendar The Ethiopian year on which this calendar is based is characterized by a rich tradition of secular events and feasts prescribed by the two major religions in the country Orthodox Christianity and Islam This tradition was well known and faithfully adhered to by the women in the present survey Further in the culture of the country the menstrual cycle is accorded special attention as it is associated with deepseated religious beliefs and widely observed rituals related to social conduct food preparation and the like However as in the case of other similar studies neither the validity nor the reliability of our interview method has been properly tested Post menstrual age values were used when the mother was sure of her dates i e within ±3 days

Anthropometric measurements The newborn infants were examined by experienced nursing staff of the centers after special training and following a thorough review of procedures Anthropometric data on the newborns were collected as a rule immediately after delivery using standard procedures (16) Weight was recorded to the nearest 10 g on carefully adjusted infant scales Length measurements were performed on standard boards to the nearest 0 1 cm The head circumference was measured by fiber glass measuring tapes to the nearest 0 1 cm

Determination of family income Information regarding income and socio economic status was obtained from the mother on admission to the maternity ward A questionnaire developed by ENI over a number of years was used for this purpose The method is acceptable in the case of higher income ranges where the family head earns a fixed regular income but provides only approximate figures for low income groups which show considerable social mobility and in most instances have temporary employment An income above Ethiopian $600 per month (Eth $100=US$ 49) corresponds roughly to the starting salary of a recent university graduate (30) A monthly income not exceeding Eth $100 is known to be inadequate for the purchase of a balanced family diet (unpublished observation ENI)

Data processing and statistical procedures As part of the survey a number of additional variables such as maternal age parity and maternal body weight and height were also recorded At the time of writing however only

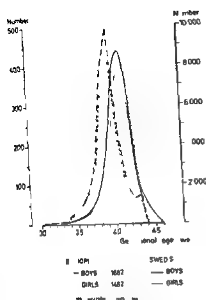


Fig 1 Distribution of Ethiopian infants by gestational age compared with that observed in Swedish infants (33)

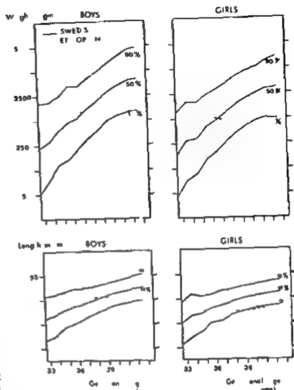


Fig 2 Birth weight and birth length in relation to gestational age in Ethiopian infants compared with a Swedish standard (33)

the data presented in this paper could be obtained the rest being inaccessible due to the current civil unrest in Ethiopia Consequently a number of analytical procedures considered important in the development of optimal intrauterine growth standards (35) have had to be postponed for the time being Thus the distribution of birth weights at each post menstrual age has not been examined for normality it has not been possible to plot the first born separately from infants born later and finally no correction has been made for maternal age and size or parity Additional measures of maturity are not presented and no information on the postnatal growth of the infants is available In spite of this it was felt worthwhile to report our observations

The mean and median birth weight length and head circumference and their standard deviations were determined for each week of gestation Plots of birth weight and length compared to gestational age were prepared using median values after first adjusting these by means of moving averages The age scale is indicated as 34+ 35+ 36 and so on meaning that a child said to be born at 34 weeks was born any time between 34 0 and 34 99 weeks

For comparison the results are evaluated against a Swedish reference material (37 33) one of a few recognized standards for intrauterine growth (35)

RESULTS

A total of 3 144 infants (1 682 boys and 1 462 girls) born at both institutions were recruited Tables 1–3 show the mean the standard deviation the median and the smoothed median for birth weight birth length and head circumference for each sex distributed by week of gestation (11 boys and 21 girls below 34 weeks of gestation and 6 boys above 43 weeks not included)

Birth weight birth length and head circumference Table 4 gives the mean and S D for gestational age birth weight birth length and head circumference for the Ethiopian newborns On the average Ethiopian boys weighed approximately 400 g and girls 300 g less than Swedish newborns The mean birth length of Ethiopian boys was 1 7 cm shorter and of girls 1 9 cm shorter than figures reported for Swedish infants The mean head circumference for boys was 1 5 cm smaller and for girls 1 0 cm smaller in the Ethiopian newborns than a North American standard (23)

Distribution of infants by gestational age The distribution of the infants by gestational

Table 2 *Birth length means median and standard deviation by sex and gestational age*

Gestational age (weeks)	No of cases	Mean length (cm)	S D (cm)	Median length (cm)	Smoothed median length (cm)
Male					
34	28	49 7	1 52	50 [illegible]	49 4
35	45	49 5	2 28	50 5	50 6
36	81	49 4	1 89	50 7	50 6
37	158	49 4	2 05	50 [illegible]	50 6
38	383	49 5	1 98	50 6	50 6
39	493	49 8	2 04	50 7	50 7
40	239	49 9	2 39	50 8	50 8
41	97	50 1	1 61	50 [illegible]	50 8
42	65	50 0	1 52	50 8	50 8
43	76	50 0	1 81	50 8	
Female					
34	24	49 0	1 91	49 6	50 1
35	47	49 3	2 36	50 2	50 1
36	80	49 3	1 71	50 5	50 0
37	118	48 8	1 67	49 4	49 8
38	343	49 2	1 72	49 4	49 7
39	445	49 4	2 19	50 2	50 1
40	211	49 6	2 11	50 6	50 4
41	72	49 8	1 55	50 3	50 [illegible]
42	40	49 8	1 06	50 8	50 7
43	61	50 1	1 40	50 9	

Table 3 *Head circumference means median and standard deviation by sex and gestational age*

Gestational age (weeks)	No of cases	Mean H C (cm)	S D (cm)	Median H C (cm)	Smoothed median H C (cm)
Male					
34	28	33 7	1 54	34 1	33 9
35	45	33 6	1 34	34 8	34 6
36	81	33 8	1 75	34 8	34 8
37	158	33 8	1 52	34 9	34 6
38	383	33 7	1 40	34 0	34 4
39	493	33 8	1 74	34 3	34 [illegible]
40	239	33 9	2 77	34 4	34 4
41	97	34 0	1 44	34 6	34 6
42	65	34 3	1 25	34 7	34 7
43	76	34 2	1 37	34 9	
Female					
34	24	33 1	1 37	33 6	33 7
35	47	33 8	2 58	34 0	34 2
36	80	33 4	1 38	35 1	34 4
37	118	33 5	2 10	34 1	34 7
38	343	33 7	1 92	34 9	34 3
39	445	33 8	2 05	34 0	34 3
40	211	33 6	1 32	34 1	34 1
41	72	33 9	1 32	34 1	34 4
42	40	33 4	1 30	34 9	34 6
43	61	33 7	1 67	34 8	

Table 4 *Birth weight length and head circumference of Ethiopian infants compared with Swedish values (32 33)*
Mean ± S D are indicated

	Ethiopia		Sweden	
	Boys	Girls	Boys	Girls
Length of gestation (days)	274 1±13 6	273 3±13 7	281 ?±13 5	?81 2±13 3
Birth weight (g)	3 185±465	3 084±411	3 ?96±54?	3 408±503
Length (cm)	49 7±? 0	49 4± ?8	51 4±? 4	?0 3±2 3
Head circumference (cm)	33 8±1 8	33 7±1 8	35 3	34 7

(33) (?3)

age is given in Fig 1 The mean length of gestation was 274 1 (S D 13 6) days for boys 273 3 (S D 13 7) for girls and 273 8 (S D 13 6) for the total The mean for the Ethiopian infants was 7 4 days shorter than that reported for Swedish infants 10 9% were born before the 37th week of gestation

Birth weight and birth length in relation to gestational age Fig 2 shows the pattern of anthropometric growth between the 34th and 43rd weeks of gestation The median values for weight and length show a tendency to be higher in Ethiopian infants up to about the 34th–35th weeks of gestation After that time the pattern is reversed with almost a complete standstill in growth in both anthropometric variables among Ethiopian newborns

Distribution of birth weight by family income The birth weight of the infants showed a significant upward trend with increasing income ($p<0.05$) (Fig 3) Infants born to mothers from the lowest income group were on the average close to 500 g lighter than those of the highest income group who were comparable to Swedish infants

Distribution of birth weight by season Fig 4 shows the distribution of mean birth weights by season for one of the centers which mainly

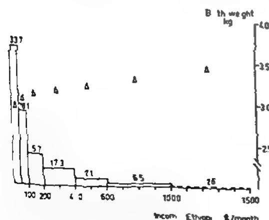


Fig 3 Distribution of birth weight of Ethiopian infants by family income Triangles indicate mean birth weights Bars represent percentage distribution of family income

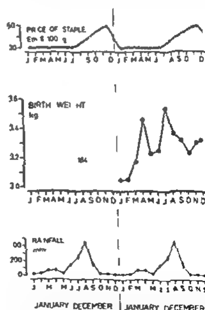


Fig 4 Distribution of birth weight of Ethiopian infants by season compared with approximate average price for one major staple (tef) and the average monthly rainfall in Addis Abeba Birth weight data available only for one year

...ters for the indigent with approximate average price for one major staple (tef) and the average monthly rainfall in Addis Abeba. The mean birth weight seemed to show a seasonal variation with a significant upward trend ($p < 0.05$) between January and July. The lowest mean birth weights were registered for the months of January, February and March for pregnancies commencing around April, May and June. For these pregnancies the third trimester coincided with the pre-harvest season when staple foods are scarce and prices are customarily at a maximum.

DISCUSSION

In the present study, as in the Swedish investigation, the duration of pregnancy was calculated from the last menstrual period, a method found to be the best available in validity tests (13). Our findings with use of the interview method may be expected to produce valid results, since the menstrual cycle, which still retains cultural significance, is recollected by the mother with relative ease and hormonal contraceptives which cause withdrawal bleeding that can be mistaken for true menstruation (26) are rarely used in the population under survey. On the other hand it is believed that this method overestimates fetal age by about fourteen days (3). Since the interval from the first day of the last menstrual period until conception or the onset of cell multiplication is possibly variable (15), attempts to define a "true" gestational age imply a precision which is not valid. We have no accurate information on the usual length of the menstrual period in Ethiopian women and the distribution of gestational age in the population under study has not been investigated earlier.

The mean length of gestation of 273.8 days is 7.4 days shorter than the Swedish one, and 5–6 days shorter than the mean for Taiwanese babies (19), but is similar to the 274.5 days reported for a large number of Hong Kong newborns (27). In studying newborn infants in Gothenburg, Sweden and Palermo, Italy, Fryer et al. (9) have recently reported a difference between the median gestational age for the two populations of the order of 4 days, the gestational period being shorter in Palermo. Available information regarding the average duration of gestation in different human groups indicates that the variation is small between social classes or ethnic groups and is considered to be due to differences in the interview methods used to determine gestational age (14).

Even extreme situations such as famine of sudden onset, although they affect birth weight, would seem to have only minor influence on the length of gestation in a population. Sindram (28) reported a mean reduction in gestational length by only four days during the Dutch famine. In a review of the data from this winter famine of 1944–45 published recently, Stein et al. (31) did not observe any statistically significant reduction in the length of gestation in affected women. A slight contraction of the period of gestation was observed in the cohort exposed to famine only in the first trimester.

Reports from chronically malnourished communities are even more sparse and none exist for Ethiopia. A WHO Expert Committee (39) observed marked differences in the mean birth weights in a number of countries at different stages of development, although the mean length of gestation did not differ significantly. Conversely, secular trends in birth weight detected in a number of populations (8, 12) have not been accompanied by a corresponding increase in length of gestation, which has remained constant.

We have no explanation for the shorter gestational age in the Ethiopian population compared with the Swedish. For some of the reasons mentioned above the results must be interpreted with caution. The observed shortened gestation could depend on the introduction of a systematic error whereby the last day of the last menstrual period might have been used in determining duration of pregnancy instead of the first day. This is unlikely, how-

ever as specific expressions were used to differentiate between these two dates and special care was taken to register the information in separate columns

One effect of this shift in the distribution of gestational length to lower values (Fig 1) is an increase in the preterm rate among our newborns Of these 10 9% were born before the 37th week of gestation a rate that is more than twice as high as that reported from industrialized countries e g Great Britain (4 9%) (4)

It is difficult to evaluate the impact on birth weight of a shortening of gestation of the order observed in this study Fetal growth appears *to have its maximum velocity in respect* of weight between the 32nd and 38th weeks of gestation By 39 weeks an asymptote is reached However the mean birth weight and possibly also the growth velocity at different gestational ages show variations in different countries and even districts (4 5 20 38) We have found limited information on this matter from developing areas Data collected from five investigations in industrialized countries (5) indicate birth weight ranges of 1 020–1 236 g at 28 weeks and 3 226–3 500 g at 40 weeks with the Swedish mean remaining *the highest* Using the Swedish figures a maximum weight deficit of 200 g can be expected if the length of gestation is shortened by seven days from the norm

In the present series a plateau is already attained by 36 weeks after which the intrauterine increase in weight and linear growth has practically ceased (Fig 2) Consequently shortened gestation alone cannot explain the difference in mean birth weight observed between the Ethiopian and Swedish infants In fact although there is an increase in the preterm rate in our material the proportion of small for gestational age preterm neonates using the Swedish 10th percentile as standard is seemingly not higher (Fig 2) If anything the Ethiopian infants display a rate of growth before 35 weeks that is comparable with the Swedish norm This conforms with one observation—that American infants of African origin were larger than infants of Caucasian background when born before less than 35 weeks (24) Similarly no significant excess of premature infants of very low birth weight was observed among births conceived during the Dutch famine (20) In this regard our analysis concords with reports which indicate that practically the entire variation in birth weight among population groups could be accounted for by causes other than shortened gestation (34)

The mean birth weight for the total material of 3 139 g recorded in the present survey is practically identical with two other published *figures for* Addis Abeba infants 3 120 g $n=500$ (41) and 3 132 g $n=8469$ (11) respectively Although problems of comparability are great these are higher than reported values for the majority of other African and Asian newborns (22) for whom the birth weight approximates 2 700 g but are close to 400 g lower than West European norms where the body weight means are approximately 3 500 g (23)

Variation in birth weight which is not explained by an obvious cause such as a prenatal complication (1 7) or multiple pregnancy (4 21) has a complex etiology Several investigations have reported that the average birth weight is lower among the poorer sections of any community (39) This was also the case in the present study (Fig 3)

A number of studies have indicated that the mean birth weight differs between different ethnic groups (2) On the other hand there is considerable evidence (6 37) to suggest that privileged classes in any ethnic group can produce infants with birth weights very similar to those of Swedish or other Caucasian infants This conforms with our finding that the newborns of the lowest income group were close to 500 g lighter than those of highest income group but the difference between the latter and the Swedish norm was only slight (Fig 3)

It has been suggested that the birth weight decreases as the altitude increases (18) and

that intrauterine growth in late gestation may be slower in hotter months (27) Addis Abeba is situated about 2500 m above sea level and has an even daily temperature range of approximately 20–30°C throughout the year However these environmental factors may not account for more than a few per cent of the total variance in birth weight (27)

The explanation for the observed growth pattern *in utero* is not immediately apparent The mean anthropometric values at birth are attained by a retardation of growth rate occurring late in pregnancy and are seemingly not the result of a uniform process of slow growth along a lower centile Although the weight–length ratio at each gestational age has not been investigated both weight and linear growth seem to be affected in the same proportion (Fig 2)

In addition to the combined influence of environmental and maternal factors gestational malnutrition has been implicated as a major cause of LBW (2) Size at birth has been shown to decrease as a result of acute severe food shortage during the third trimester (31) No added effect from exposure during the first and second trimesters was apparent Recently Lechtig et al (17) have reported an increase in birth weight following energy supplementation during pregnancy among a population in which mild moderate malnutrition is endemic

A previous study in Addis Abeba (10) of the dietary intake in the third trimester of pregnancy has shown that non privileged women consume a monotonous diet with an average intake of energy, protein and nutrients that is markedly below the FAO/WHO Recommendations (25) whereas the privileged women have a varied menu which also includes ample quantities of animal protein and fresh fruit These findings and the observed seasonal variation in mean birth weight suggest a third trimester effect of food shortage on intrauterine growth in our population (Fig 4) Although the possible effects of other factors such as subclinical intrauterine infection have not been fully investigated and a specific indicator is lacking late gestational malnutrition either of energy and protein or of specific nutrients appears to be a plausible explanation for the growth retardation *in utero* observed among Addis Abeba infants Further follow up surveys will help to identify the relative importance of the various causative factors against which preventive action can be taken

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CASE REPORT

JUVENILE CIRRHOSIS AND MEMBRANOUS GLOMERULONEPHRITIS IN A CHILD WITH ALPHA$_1$ ANTITRYPSIN DEFICIENCY PiSZ

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ABSTRACT Rodriguez Soriano J Fidalgo I Camarero C Vallo A and Oliveros R (Departments of Paediatrics and Pathology Hospital Infantil de la Seguridad Social Bilbao Spain) Juvenile cirrhosis and membranous glomerulonephritis in a child with alpha anti trypsin deficiency PiSZ Acta Paediatr Scand 67 793 1978 —An infant with alpha anti trypsin (α AT) deficiency PiSZ presented with liver cirrhosis and showed clinical and labora tory evidence of renal disease when hepatic decompensation developed shortly before death at 12 months of age Low serum levels of α AT were only demonstrated late in the disease SZ phenotype was proved by starch gel electrophoresis Post mortem pathological studies re vealed sevre hepatic cirrhosis with intracytoplasmic inclusion of α_1 AT and membranous glomerulonephritis with deposits of complement and immunoglobulins but without the pres ence of α AT The present case suggests the importance of studying Pi phenotypes and serum levels of α AT in all cases of idiopathic cirrhosis or renal disease in infancy

KEY WORDS Alpha antitrypsin deficiency hepatic cirrhosis membranous glomerulonephritis

Severe hereditary deficiency of alpha$_1$ antitrypsin (α_1 AT) PiZ has been clearly as sociated with pulmonary and hepatic disorders in adults (1 7) and with neonatal hepatitis and cirrhosis in children (9 11–14) Other clinical manifestations recently reported are membranoproliferative glomerulonephritis (10) and pancreatic fibrosis with glucose in tolerance (6) The association of disease with protease inhibitor (Pi) phenotypes other than PiZ (MZ FZ SS MS SZ) is uncertain and only a few such cases have been reported Hepatic cirrhosis has been observed how ever both in children and adults with FZ (1 2) and SZ (3–5 11 15) Pi phenotypes

This report presents the clinical and path ological findings in an infant with α_1 AT defi ciency PiSZ who presented with progressive liver cirrhosis and later developed membran ous glomerulonephritis

CASE REPORT

This male infant was examined at 45 days of age because of jaundice persisting since the first day of life Physical examination revealed a well nourished but jaundiced in fant with marked hepatosplenomegaly Weight was 4 1 kg and length 57 cm The results of liver tests are sum marized in Table 1 Serologic investigation of lues toxo plasmosis cytomegalic virus disease rubella herpes sim plex and hepatitis B were negative Total serum protein electrophoretic fractions and immunoglobulin levels were normal Sweat chloride concentration was in the normal range Reducing substances could not be found in the urine Blood and urine cultures were also negative Radiological studies of esophagus kidneys and skeleton were normal The patient was discharged when 70 days old with the diagnosis of "neonatal hepatitis syndrome of unknown etiology

Follow up showed persistence of jaundice and progres sive clinical signs of hepatic cirrhosis with evidence of portal hypertension and ascites Rickets became apparent but healed with parenteral administration of 200 000 I U of vitamin D A liver biopsy performed at 6 months of age revealed marked fibrosis biliary duct proliferation and biliary plugs PAS Diastase positive intracytoplasmic in clusions were not observed

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Necropsy findings

Lung Massive pulmonary hemorrhage without inflammatory signs

Liver On gross examination the surface was greenish irregular and finely granular Histologically the normal liver structure had disappeared and extensive bands of fibrosis delineating regenerative nodules of variable form and size were observed Biliary plugs were present Hepatocytes contained intracytoplasmic inclusions which stained positively with PAS Diastase and showed strong fluorescence with FITC labeled α_1 AT antiserum

Kidneys They were normal on gross examination Histologically the glomeruli showed diffuse involvement with collapsed capillaries eosinophilic areas without nuclei marked thickening of basement membranes and focal and mild mesangial proliferation They showed a grossly granular pattern of fluorescence along capillary walls when incubated with FITC labeled antisera to IgA IgM and C_3 but not to IgG fibrinogen or α AT (Fig 1)

COMMENTS

Severe hepatic involvement has rarely been reported in children with PiSZ phenotype Clinical descriptions include a neonatal hepatitis syndrome (11 15) hepatomegaly with or without elevation of hepatic enzymes in the serum (4 11) and onset of portal hypertension during early childhood (5) In our patient hepatic disease was specially severe since it manifested from birth as a neonatal hepatitis syndrome and progressed to early development of cirrhosis Although the serum level of α_1 AT was moderately depressed at the time of the initial investigation a liver biopsy performed at 6 months of age did not reveal the presence of intracytoplasmic inclusions and this led to a delay in establishing the diagnosis However a relationship between liver damage and α_1 AT deficiency was later demonstrated by the steady decrease of the serum levels of α_1 AT as well as the presence of inclusions of the protein in necropsy material

The association between renal lesions and α_1 AT deficiency is a recent discovery Miller & Kushner (8) found proliferative glomerulonephritis and necrotizing angiitis in an adult with emphysema and α_1 AT deficiency PiZ Moroz et al (10) found membranoproliferative glomerulonephritis at post morten examination of three PiZ children with α_1 AT deficiency who died of cirrhosis Renal involvement became evident at the time of decompensation of the liver disease Immunofluorescence studies and electron microscopy performed in one patient revealed subendothelial deposits of α_1 AT C3 and immunoglobulins along the basement membrane Renal involvement in our case was markedly similar It manifested itself also at the time of decompensation of cirrhosis but in contrast to the case of Moroz et al the mesangial proliferation was mild and no deposits of α_1 AT could be demonstrated in the glomeruli To our knowledge this case represents the first description of immunological renal disease in a PiSZ individual

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The authors thank Dr P J Cook of the Department of Human Genetics University of London for studying the phenotype of α AT

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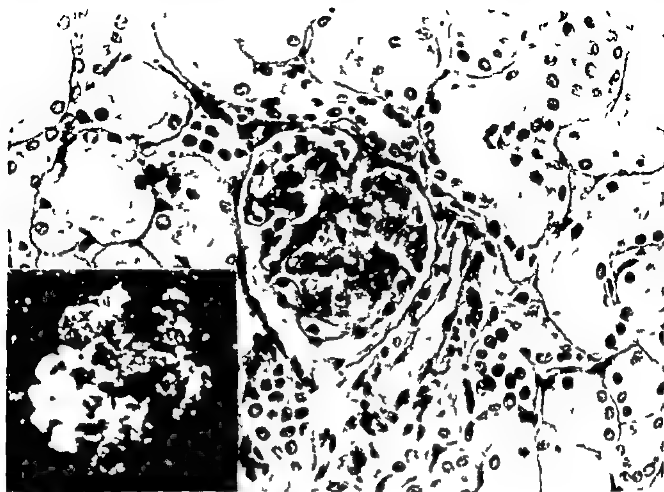


Fig 1 Glomerular changes with collapsed capillaries nuclei free dense eosinophilic areas and thickened basement membranes (HE ×125) *Insert* immunofluorescence with FITC labeled C3 antiserum (×125)

During the subsequent 6 months he showed progressive malnourishment and steatorrhea A steady decrease in the serum level of α_1 AT was evident and at 10 months of age a value of 40% of normal was measured PiSZ phenotype was demonstrated by starch gel electrophoresis At 12 months of age following an episode of acute gastroenteritis he developed hepatic coma which subsided with standard supportive measures At this time he presented generalized edema oliguria proteinuria (1/mg/m²/hr) hypoproteinemia and hypoalbuminemia He died with signs of pulmonary edema after 2 weeks of hospitalization

No evidence of lung or liver disease was found in any of the family members

Table 1 *Biochemical findings*

Age (months)	2	6	12	Normal values in children
Total bilirubin (μmol/l)	126	98	210	up to 12
SGOT (μmol s⁻¹/l)	0 45	0 26	0 26	0 08–0 17
Cholesterol (mmol/l)	13 1	6 6	7 2	3 9–5 2
Prothrombin (%)	100	82	36	>80
α_1 antitrypsin (%)	65	47	26	>75[a]
C3 (% normal adult pool serum)	–	–	60	113±26[b]
C3 Activator (% normal adult pool serum)	–	–	50	107±23[b]
C4 (% normal adult pool serum)	–	–	38	84±24[b]
C5 (% normal adult pool serum)	–	–	83	118±19[b]

[a] 100% corresponds to 2 g/l
[b] Normal values (mean ± 1 S D) in a control group of children studied in our laboratory

Necropsy findings

Lung Massive pulmonary hemorrhage without inflammatory signs

Liver On gross examination the surface was greenish irregular and finely granular Histologically the normal liver structure had disappeared and extensive bands of fibrosis delineating regenerative nodules of variable form and size were observed Biliary plugs were present Hepatocytes contained intracytoplasmic inclusions which stained positively with PAS Diastase and showed strong fluorescence with FITC labeled ■ AT antiserum

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CASE REPORT

ABETALIPOPROTEINEMIA TREATED WITH PARENTERAL AND ORAL VITAMINS A AND E AND WITH MEDIUM CHAIN TRIGLYCERIDES

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ABSTRACT Azizi E Zaidman J L Eshchar J and Szeinberg A (Departments of Pediatrics A Biochemical Pathology and Institute of Gastroenterology Asaf Harofe Hospital and Department of Chemical Pathology Chaim Sheba Medical Center The Sackler School of Medicine Tel Aviv University Zerifin Israel) Abetalipoproteinemia treated with parenteral and oral vitamins A and E and with medium chain triglycerides Acta Paediatr Scand 67 797 1978 —An 11 year old girl with abetalipoproteinemia was treated with parenteral vitamin A and vitamin E for two and a half years Some improvement in neurological and visual deficits was noted On changing to oral vitamin E and later with addition of medium chain triglycerides (MCT) to the diet a considerable improvement in her general wellbeing neuromuscular lesions and ophthalmological symptoms was noted This regimen is being adhered to for five and a half years The condition is stable with no further improvement

KEY WORDS *Abetalipoproteinemia vitamin A vitamin E MCT*

Abetalipoproteinemia or Bassen–Kornzweig syndrome (2 35) is a rare autosomal recessive disease (14) The basic biochemical defect is the absence of low density lipoproteins (19) possibly due to a defect in the apoprotein (5 26) The clinical features include acanthocytosis malabsorption pigmentary retinopathy and atactic neuropathy Growth failure is prominent in childhood In adolescence the neurological abnormality results in progressive disability Death has been reported in early adult life usually from cardiac involvement Specific treatment is not yet possible (11 15 17 22) but symptomatic therapy with a low fat diet and supplementary oral vitamins has been described (23) We report the successful use of a combination of vitamin A vitamin E and medium chain triglycerides (MCT) A preliminary communication concerning this patient has already been made

METHODS

Blood samples were taken after an overnight fast and allowed to clot The sera were adjusted with 0 01% EDTA Na and lipoprotein fractions were isolated by preparative ultracentrifugation (8) The isolated fractions were dialysed against 0 9% NaCl–0 01% EDTA Na_2 Electrophoresis of lipoproteins was performed on cellogel strips (Chemetron Milano) Immunodiffusion assays (27) were performed using specific antibetalipoprotein serum (Miles Laboratories Rehovot Israel) The various serum samples and lipoprotein fractions were analyzed for total lipids (30) triglycerides (36) total cholesterol (18) phospholipids (31) vitamin A (34) and vitamin E (20) Medium chain triglycerides (Mead Johnson) used in this work is composed mainly of C and C fatty acid esterified with glycerol

CASE REPORT

The patient is a Jewess of Turkish origin Her parents are first cousins and her two siblings are healthy She was first admitted to hospital at the age of 3 5 months because of prolonged diarrhea For the next ten years she continued to have diarrhea and repeated infections of the respiratory tract some of them severe A gluten free diet was associated with a limited improvement of the diarrhea Abnormal neurological features were first noted at

Table 1 *Analysis of serum total lipids non esterified fatty acids phospholipids triglycerides, cholesterol lipoproteins and vitamin E in patient family and control*

TL=total lipids NEFA=non esterified fatty acids PL=phospholipids TG=triglycerides TC=total cholesterol VLDL=very low density lipoprotein ($D<1\ 006$) LDL=low density lipoprotein ($1\ 006<D<1\ 063$) HDL=high density lipoprotein ($1\ 063<D<1\ 210$)

Subject	Age (years)	Serum TL (g/l)	Serum NEFA (μmol/l)	Serum PL (mmol/l)	TG (mmol/l) Serum	TG VLDL	TG LDL	TG HDL	TC (mmol/l) Serum	TC VLDL	TC LDL	TC HDL
Patient	11	1 99	39	0 7	1 2	0	0	0 8	1 6	0	0	0 9
Brother	25	7 0	190	3 7	9 15	5 5	2 9	0 9	5 3	0 5	3 4	1 3
Sister	9	5 2	195	3 6	8 6	4 4	3 1	1 0	4 8	0 7	3 3	0 9
Mother	42	6 5	156	2 5	9 4	5 5	2 4	0 8	5 5	0 9	4 1	1 0
Father	46	6 9	201	3 3	12 4	6 4	3 2	1 1	4 9	0 7	3 0	1 2
Normal female	36	5 85	226	–	7 9	4 5	1 9	0 9	5 3	1 3	2 5	1 5
Normal male	38	6 1	236	–	11 0	6 8	3 4	1 1	5 8	1 3	2 9	1 5
Normal range												
Low		4 0	108	1 9	3 2				3 9			
High		10	542	3 9	16 4				7 3			

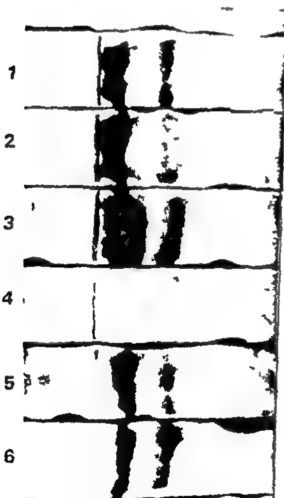


Fig 1 Cellogel lipoprotein electrophoresis of serum 1 patient s brother 2 sister 3 father 4 patient herself 5 her mother and 6 normal control

the age of nine years At the age of 11 years she fell and fractured her pubic bone and at that time acanthocytes were described in a smear of her peripheral blood Other hematological findings including an abnormal hemoglobin were also seen and already described (10) Her neurological disorder had advanced with winging of the scapulae absent knee jerks atrophic shoulder girdles and atrophic pseudohypertrophy of calf muscles bilateral foot drop an atactic gait and a lumbar hyperlordosis Coordination was slightly affected Some atrophy of the trapezius and weakness of serratus anterior and deltoid muscles were also present EEG and EMG were within normal limits Muscle biopsy revealed non specific myopathy lack of striation eosinophylic cytoplasma phagocytosis and some regenerating fibres The patient was night and color blind and had constricted visual fields myopia and lesions of the extraocular muscles The ERG was consistent with tapetoretinal degeneration Fecal fat was 10 g/24 hours A small intestinal biopsy showed the characteristic large fat vacuoles in the jejunal mucosa The diagnosis was confirmed by demonstrating absence of prebeta and betalipoproteins in the serum by cellogel electrophoresis and by immunodiffusion (Table 1 Figs 1 and 2) The levels of vitamin E in the patient s serum and in a control serum and their distribution in different ultracentrifugal fractions are shown in Table 2 Serum levels of vitamin A were subnormal and ranged between 0 73 to 1 05 μmol/l (Normal values in our laboratory are 1 75–7 0 μmol/l)

Treatment was started with alpha tocopherol acetate 0 1 g i m twice weekly and vitamin A 150 000 u i m once weekly for 10 months Since that time and for one and a half years vitamin E was given once weekly only and vitamin A once a month Serum levels of vitamin E ranged between 3–3 5 mg/l during the period of treatment Serum levels of vitamin A ranged between 1 4–2 5 μmol/l Physical examination at this stage showed considerable

itamin E mg/l			
Serum	VLDL	LDL	HDL
1 3	[illegible]	0	0
10 5	[illegible]0	[illegible]	3
11 [illegible]	1 0	7 0	3 5
11 5	0	8 8	[illegible]0
11	0	8 5	3 [illegible]
[illegible]	0	9 3	2 0
13	1 0	8 6	3 5
8 3			
15 4			

improvement in the motor function of the muscles The atactic gait disappeared almost completely and coordination returned to normal Muscle biopsy at this stage was normal Because skin necrosis developed at the site of vitamin E injection and as Muller et al (25) reported success with oral therapy the route of alpha tocopherol acetate administration was changed to oral intake of 3 g daily No deterioration in neurological features was noted but neither was there further improvement during six months of this treatment Serum levels of vitamin E decreased from 3–3 5 mg/l to 1–3 mg/l At this stage 40–50 g MCT were added daily and oral vitamin E as well a i m vitamin A were continued Her diet was never limited After six further months on this combined regimen the patient gained 3 5 kg in weight Further improvement in her general wellbeing was observed The patient is no more atactic and motor function of the muscles is normal She is able to walk freely in twilight as well as in darkness However there is no change in the ERG findings MCT was given for two years Nine months later neurological and ophthalmological examinations were unchanged

In summary the girl was treated for 28 months with parenteral vitamins A and E 6 months on oral administration of both vitamins Twenty four months with added MCT and until now 9 more months without MCT but still on oral intake of both vitamins total duration of therapy being 67 months

DISCUSSION

In abetalipoproteinemia there is malabsorption of vitamin E possibly due to failure of chylomicron formation (24) This malabsorption is one reason for treating these patients parenterally or orally in large doses (25) Another reason for administering tocopherol are the neuromuscular disturbances seen in this disease in view of the findings that tocopherol helped animals with muscular dystrophy (4 33)

Normally vitamin E is found to be associated with the low density lipoprotein (LDL) fraction in the fasting state (1 28) and with the chylomicrons in induced hyperlipidemia (32) and is usually carried in the blood by the beta lipoproteins (LDL) (12 21 28) In the absence of LDL the vitamin may be carried by albumin (37) This probably is how these patients including our case are able to carry and utilise exogenous vitamin E Indeed our control case had 60% of ingested vitamin E in the LDL fraction (Table 2)

However half of the patient s vitamin E after parenteral loading was recovered in the HDL and half found in the albumin fractions

Vitamin A was administered to our patient in an attempt to alleviate some of the ophthalmological problems The underlying cause of retinitis pigmentosa in abetalipoproteinemia is unknown (6) Treatment with vitamin A alone was uneffective (39) Successful therapy should possibly consist of a combination of vitamin A vitamin E and the essential fatty acid linoleic acid Our patient improved while

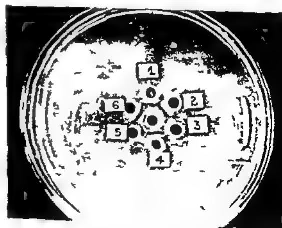


Fig 2 Immunodiffusion of serum The center well contains antibetalipoprotein antibodies 1 patient s brother 2 patient s sister 3 patient s father 4 patient s mother 5 patient herself and 6 normal control

Table 2. *Vitamin E in the serum of the patient and control before and after vitamin E loading*
All values are mg/l. a = immediately before 0.1 g vitamin E i.m. b = three hours later

	Total in serum	VLDL	LDL	HDL	Albumin
Patient					
a	1.7	0	0	0	1.5
b	7.4	0	0	3.8	3.9
Control					
a	12.5	0	9.5	3.2	0
b	21.6	2.2	12.4	4.8	2.0

on a combined regimen of vitamin A, vitamin E and MCT, which, however, does not contain linoleic acid.

The addition of MCT to the patient's diet is recommended (7), though the mechanism by which it helps is uncertain (3, 7). The object of using MCT is that these triglycerides are not transported as chylomicrons but are albumin bound. For that reason MCT can be absorbed and reach the liver via the portal system. In our patient, as in one of two patients described by Isselbacher et al. (9), addition of MCT caused an acute, though limited, rise in the patient's weight. The gain in body weight by the use of MCT was quite expected and is possibly due to the increased uptake of lipids. We are unable to relate the improvement in our patient's gait, vision and neuromuscular status to a direct effect of MCT. The possibility of hepatic lesions during prolonged MCT therapy was recently described (29). Our patient was closely followed during her 2½ years of MCT therapy. No hepatomegaly, neither disturbances in liver function tests, transaminases or alkaline phosphatase were noted. Thus we thought percutaneous liver biopsy to be ethically unwarranted. Another possible hepatotoxin is vitamin A (13). Fortunately this agent too did not effect our patient's liver.

In abetalipoproteinemia, as in other cases of fat malabsorption, it is advisable to limit oral fat intake (29). However, in this disorder it is not a prerequisite, especially not in older age groups. Accordingly this was not done in the present case. Instead the patient received MCT which contains special fats absorbable despite apolipoprotein B deficiency. Our patient's condition did not worsen during these five years of treatment and possibly even improved. Although a chance occurrence cannot be excluded, it is likely that this benign course is the result of the combined management with vitamin A, vitamin E and MCT.

ACKNOWLEDGEMENT

Our thanks are due to Prof. J. K. Lloyd from St. George's Hospital, London, for her many comments and remarks. We also thank Dr R. Spira, from Asaf Harofe Hospital, for the EMG examination, Prof. A. Auerbach from Hadassah Medical School, Jerusalem, for the ERG examination, to Dr G. Neumann of the Sheba Medical Center, for the small bowel biopsy and to Mrs A. Yulsari, M.Sc. of the Asaf Harofe Hospital for the lipid determination.

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BOOK REVIEWS

R Bernbeck & G Dahmen *Kinderorthopädie* Georg Thieme Verlag Stuttgart 1976 563 pp illus DM 178 ISBN 3 13307307 8

The principles of treatment in this book are primarily derived from the Munich school the most well known representatives of which are Hohmann and Lange

The literature references in some chapters are almost exclusively from German journals As present day Scandinavian paediatric orthopaedics also has strong connections with Great Britain USA and Canada many of the principles of treatment are unfamiliar to a Swedish orthopaedic surgeon Some of them are based on personal views and some appear old fashioned In Perthe s disease for example Catterall s prognostic classification in groups and head at risk changes is not mentioned

The presentation is not very fluent and it is difficult to read long parts at a time In places the authors have put too much stress on trifles Thus in the description of examination techniques more than 40 measurements of different lengths and circumferences are recommended which could be taken as thoroughness but which appears unnecessary The same can be said about 39 localizations of aseptic necrosis in most cases with personal names

Some topics receive too much space while others are very summarily treated While spina bifida is dealt with on 5 pages and fractures on 18 carriage abnormalities receive 73

The indications for treatment are sparsely discussed Procedures are recommended which we now regard as quite unnecessary for congenital pes calcaneo-valgus redression bandage and in some cases operation for Mb Baastrup local steroid injections and operation with extirpation of processus spinosus in resistant cases etc

The chapter on fractures is not good but the one dealing with congenital dislocation of the hip is one of the best Certainly the authors hold the view that it is due to a congenital underdevelopment of acetabulum but they share the generally accepted principles of treatment We can only be grateful that we have no need here for the 9 pelvic osteotomies which are described

In conclusion the book cannot be recommended as the first book in paediatric orthopaedics as it is inferior to Tachdjian and Sharrard s textbooks Possibly it could be used as a complement

Lars Danielsson

Carl Pochedly (ed) *Leukemia and lymphoma in the nervous system* 229 pp illus C Thomas Springfield Ill No price given

In the preface to this book the author states that this volume is intended to be an in-depth review of the various clinically relevant aspects of leukemia and lymphoma as they affect the nervous system Such a review is well motivated against the present knowledge of the crucial importance of CNS leukemia involvement for the prognosis of the leukemia During the last two decades the role of CNS involvement has changed from a matter of a final haemorrhagic complication to a specific progress of the neoplastic disease itself which limits the prospects of complete cure The book ends by suggesting a protocol for the treatment of overt CNS leukemia the goal of which is not only palliation but cure This is a new concept as one is still inclined to regard overt CNS leukemia as universally fatal This new concept however is the corollary of the detailed investigation of CNS leukemia presented in this book

The chapters are presented in such a way that they can be read as separate entities which makes the book a valuable and practical handbook Especially interesting is the profound discussion on "How does leukemia invade the CNS?"—bringing up the important question of how the CNS can offer a sanctuary to leukemic cells The clinical and pathological aspects of CNS leukemia are described against the background of normal CNS meningeal anatomy and physiology The available methods for cytological examination of CSF are presented giving the clinician much advice in diagnosis The exhaustive description of the therapeutic possibilities also contains information about the neurotoxic effects of the treatment The author thus underlines the necessity of maximally effective and minimally damaging therapeutic regimens

Although the main topic of this book is acute lymphatic leukemia the neurological complications of non lymphoid leukemia as well as of non Hodgkin lymphomas are also covered

In the reviewer s opinion this book will be of great value to the pediatrician working practically with childhood leukemia

G Bodegård

List of Supplements to Acta Pædiatrica Scandinavica

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68 Wegelius R th O th Ch ge th P riph ral Blood Pi t re f th Newborn I f t Imm d t ly aft B rth
69 Lund l Stephan C w M lk fd y ra y I f t
70 W en B rg tta P pt d Trypt C pac ty of h Dig t Gl d Newborn A C mp so betw Prem t re nd F ll-t rm I f t
71 M asik Elisabeth Übe d Ei wark g de Kalt f da pe ph Bl bild d Sä gl gs E perim t ll U t rs ch g
72 Ouila Eino St d on the C reb osp nal Fl d Pr m t l fants
73 H rv maal M kk On H morrh g f th M d ll Obl gat d th P nd R pi ory D sord rs Pr m t Inf t
74 Henel ik Le nart R spiratory Infect m ng Ch ldre m Day [redacted] sere
75 Pape F m K onpri se sa Lov sa Ch ld Hosp tal
76 Le le-Jensson J hannes Ch l N m l e E logy d Ep d m l gy Cl al M t rial from M lmöh C ty 1910–1944
77 Th P oc d e f th A h N rth P d n Cong Cope ha g A gu 13–16 1948 Ed ed by P l Druck
78 Sonnervold Olli S 6 Eff t Ch ldre St d of th Blood S g Regul t on by M f Doubl nd T pl gl cose T l ranc T t
79 Wasz Höck rt Ol Th T berc l R act Diff re Part of th Sk nd he Se t ty a Rh matoid Arth u W th Special R f nc to th Technical Error th M ntou Reaction
80 Frisell Erik S ud on Bac m B fid m H al hy Inf t A Cl Bact riolog I I gatio
81 Tähkä H fkka O th W ght nd S ru t f the Ad nal Gland nd th Fact rs Aff t g Th m Ch ld of 0–2 Years
8 Sirén Lars S ud of th R nal E ion of P Inf ncy nd Ch ldhood
83 Th P oc d g of he T h North P d n C g Stockh lm J ne 13–16 1951 Ed d by J t St öm
84 Stoell Greta Übe das Menarchealter F land sowie e g pez l len Beobach g über dam t rbundene F ktore
85 Jorup Sigvard Colonic Hyperperistalsis N rolabil Inf t Stud in So-c lled Dyspepsia m Breastf d Inf
86 Lindquist, Bertil Effect f V m D on th M tabol m of R docal ci m Ra h c R
87 Berfenstam, Ragnar Studie Blood Zinc A Clini l nd E pe men tal I tigation the Zinc Content of Pla ma nd Blood Corpuscle w h Special Ref reac to Inf ncy
88 Dam Hagen Sigrid The Prema ar Ch ld The Incid nc Ætiol gy Mortal y nd the F of th S rv or

ANNOUNCEMENTS

INTERNATIONAL STUDY GROUP ON DIABETES IN CHILDREN AND ADOLESCENTS

Diabetes in children and adolescents is one of the most frequent endocrinological diseases in this age group There is evidence that its incidence is progressively increasing Nevertheless childhood diabetes got less attention than other endocrinological diseases by both pediatricians and diabetologists The feeling that more has to be done in this field led in 1974 to a meeting in Paris of a group of pediatricians caring for diabetic children It was decided to establish an International Study Group of Diabetes in Children and Adolescents (ISGD) the aim of which is to promote collaborative efforts in clinical studies and research on the epidemiology genetics prophylaxis physiopathology medico-social problems and treatment of diabetes in children

Members of the ISGD must be medical or paramedical personnel active in the field of pediatrics and concerned with the care of diabetic children They may include pediatricians pediatric endocrinologists pediatric dieticians pediatric social workers pediatric psychologists pediatric psychiatrists etc

Meetings are held annually (1975—Petah Tikva Israel 1976—Han sur Lesse Belgium 1977—Le Mesnil St Denis France 1978—Netanya Israel 1979—to be held in Berlin FRG) and are attended by members and guests

The abstracts of the Scientific Papers are published the Acta Paediatrica Belgica and the full reports in ISGD Bulletin (available upon request) Several coope tive studies dealing with basic problems of diabe management have been established

Presently the ISGD lists 44 active members from Arg tina Belgium Canada England Finland France G many Israel Italy Norway Spain Switzerland U S and Yugoslavia

It is hoped that the number of members will incre and that international cooperation will help achieve aims of the Study Group

The aim of the Bulletin is to enable non members to acquainted with the activities of the Group and to dispe knowledge on Juvenile Diabetes

The Steering Committee H Lestradet (Presider Z Laron (Secretary General) and H Loeb (Treasurer

Secretariat Institute of Pediatric & Adolescent En crinology Beilinson Medical Center Petah Tikva Isr Tel (03) 92 51 09

Official Seat 70 rue Beaunier 75014 Paris Fran Tel 540 53 54

INTERNATIONAL WORKSHOP ON THE AT RISK INFANT

International workshop on the at risk infant will be held in Tel Aviv Israel July 25–27 30–31 1979 Registration fee until April 30 100 US$ after May 1 120 For further information write the secretary Shaul Harel Child Dev lopment Assessment Center 14 Balfour St Tel Av Israel